1258561

# THE UNITED STATES OF AMERICA

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*December 09, 2004*

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**APPLICATION NUMBER:** *60/513,355*
**FILING DATE:** *October 22, 2003*
**RELATED PCT APPLICATION NUMBER:** *PCT/US04/35363*

Certified by

Jon W Dudas

Acting Under Secretary of Commerce
for Intellectual Property
and Acting Director of the U.S.
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PTO/SB/16 (05-03)
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# *PROVISIONAL APPLICATION FOR PATENT COVER SHEET*

**This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).**

**Express Mail Label No. EL 971287301 US**

## INVENTOR(S)

| Given Name (first and middle [if any]) | Family Name or Surname | Residence (City and either State or Foreign Country) |
|---|---|---|
| Albert J. | Banes | Hillsborough, NC |
| Melissa Marie | Maloney | Carrboro, NC |

☐ *Additional inventors are being named on the _____ separately numbered sheets attached hereto*

## TITLE OF THE INVENTION (500 characters max)

**Automated System for Imaging Artificial Tissue Constructs in a Controlled Environment**

*Direct all correspondence to:* **CORRESPONDENCE ADDRESS**

☒ Customer Number **28289** → *Place Customer Number Bar Code Label here*

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☐ Firm *or* Individual Name: Webb Ziesenheim Logsdon Orkin & Hanson, P.C.

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15535 U.S. PTO 60/513355 10/22/03

## ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 2 ☐ CD(s), Number

☒ Drawing(s) *Number of Sheets* 33 ☐ Other (specify)

☐ Application Data Sheet. See 37 CFR 1.76

## METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ Applicant claims small entity status. See 37 CFR 1.27.

☒ A check or money order is enclosed to cover the filing fees

☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 23-0650

☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($): $80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

*Respectfully submitted,*

Date: October 22, 2003

SIGNATURE _____

REGISTRATION NO. *(if appropriate)*: 43,016

TYPED or PRINTED NAME: Nathan J. Prepelka

Docket Number: 717-031951

TELEPHONE: 412-471-8815

## *USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT*

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the PTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| IN RE APPLICATION OF: | ATTORNEY'S DOCKET NUMBER |
|---|---|
| ALBERT J. BANES and<br>MELISSA MARIE MALONEY | 717-031951 |

ENTITLED

**AUTOMATED SYSTEM FOR IMAGING ARTIFICIAL TISSUE CONSTRUCTS IN A CONTROLLED ENVIRONMENT**

Mail Stop PROVISIONAL PATENT APPLICATION
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

EXPRESS MAIL CERTIFICATE

"Express Mail" Label Number EL 971287301 US

Date of Deposit October 22, 2003

I hereby certify that the following attached paper or fee

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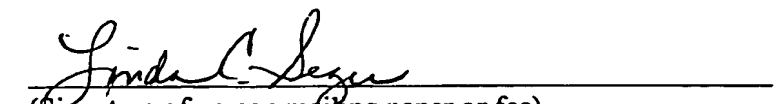
- SPECIFICATION (2 pp.)
- DRAWINGS (33) SHEETS
- CHECK FOR $80.00

is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. §1.10 on the date indicated above and is addressed to the Commissioner for Patents, Alexandria, VA 22313-1450.

Linda C. Seger
(Typed name of person mailing paper or fee)


(Signature of person mailing paper or fee)

# AUTOMATED SYSTEM FOR IMAGING ARTIFICIAL TISSUE CONSTRUCTS IN A CONTROLLED ENVIRONMENT

## BACKGROUND OF THE INVENTION

### 1. Field of the Invention

[0001] The system of the present invention is directed to a computer-implemented method and system for imaging tissue constructs in a controlled environment in the field of tissue engineering and cell biology, but could be used in any applications where programmable, automated imaging functions would be useful.

### 2. Background of the Invention

[0002] In the field of tissue engineering, changes in 3D cell-matrix constructs over time are frequently studied. Typically, the cells within these constructs will begin to form attachments within a day after plating and will reorganize and contract the matrix within a few days. Measurements of matrix contraction under the influence of various physical and biochemical factors indicate the impact of each factor on cellular function.

[0003] Currently, measurements of matrix contraction are performed manually by periodically moving the culture plates from their controlled environment (inside an incubator) to access an external imaging device (camera or scanner). Depending on the effect being measured, this process may need to be repeated every few hours, day and night, for several days. In addition to being labor intensive, this process is also less than ideal for the cell cultures themselves. Preferably, the contraction of the constructs would be monitored without repeatedly exposing the constructs to dramatic environmental changes.

## DESCRIPTION OF THE INVENTION

[0004] The system of the present invention incorporates an imaging device and a software program, which together allow the user to automatically monitor matrix contraction without removing the cell cultures from the incubator (see Figure 1). The scanner is set up inside the incubator with the cell culture plates placed on the scanner glass and the controller (computer with custom software, see Figure 2) connected and running nearby. The user inputs the desired scanning parameters (filename and type, resolution, etc), selects the scan area, and creates a scanning regimen. Once the program is launched, it will automatically initiate scans at the times indicated by this regimen, and the culture plates need only be removed from the incubator for steps such as media changes (in lengthy studies). The images are stored to a user-designated folder for future analysis or may, with additional computer processing, be automatically analyzed. Multiple imaging devices can be controlled from a

single computer/application to increase the number of culture plates that can be monitored. With the appropriate imaging devices and lighting variations (visible light, IR, UV), this application could also be extended to monitor changes at the cellular level, including performing analyses based on color distinctions. Block diagrams of the scanning function software (Figs. 3-9) and frequency set up software (Figs 10-17) are attached.

**[0005]** The computer-implemented method and automated system is embodied in the form of an executable software program, preferably with a Graphical User Interface (GUI). The user interfaces with the GUI and interacts with the method and system of the present invention. The software program of the present invention may also interact with or execute using other enabling and/or proprietary software, such as LabVIEW™ by National Instruments. Various screenshots of the present computer-implemented method, executing in conjunction with LabVIEW™, are illustrated in Figs. 18-27.

## EXAMPLE

**[0006]** Various illustrations, photographs, charts and text created in connection with the following example are illustrated in Figs. 28-34. Human tendon internal fibroblasts (HTIF, 2 x $10^5$ cells/100 µl/specimen) were plated in linear, tethered, collagen gels in TISSUE TRAIN™ culture plates. After two hours, when the gels had solidified, the culture plates were removed from the FX-4400TT TISSUE TRAIN™ Culture System and placed on the glass of a Plustek OpticPro U24 flatbed scanner. The scanner was configured to collect images every hour for the first four hours of each day and every two hours for the remainder of the day, for four days. Culture plates were only removed from the incubator once a day to change the medium. Images were imported into SigmaScan Software to quantify the area of each gel at each time point. Gels experienced the greatest rate of contraction (58.6%) in the first 24 hours, and continued contracting to a total of 73.6% by the end of Day 4. The measured contraction of the gels followed a trend consistent with contraction results obtained by traditional methods. Besides simplifying the monitoring process, however, experimental results should be more accurate with automated imaging. Any confounding results deriving from changes in temperature/pH are minimized when the cultures can remain in the incubator during imaging. Additionally, the increased number of data points collected improves the resolution of the contraction curve and strengthens the findings. This method can easily be applied to perform contraction analysis with other 3D gel systems, as well.

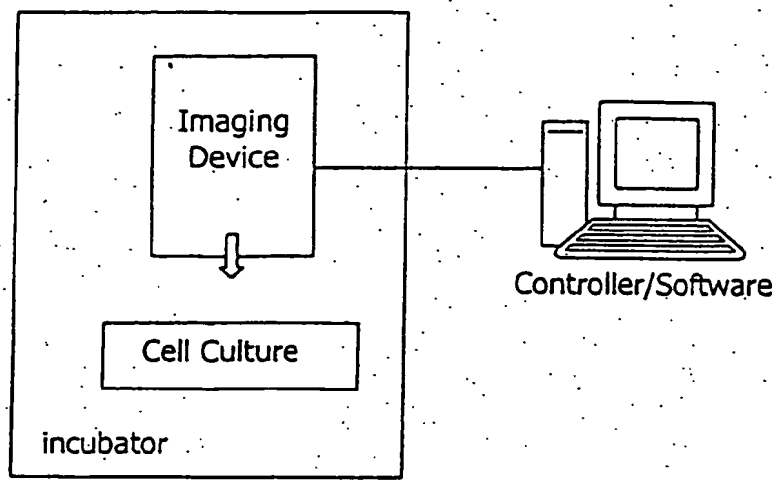



Figure 1: Components of Imaging System

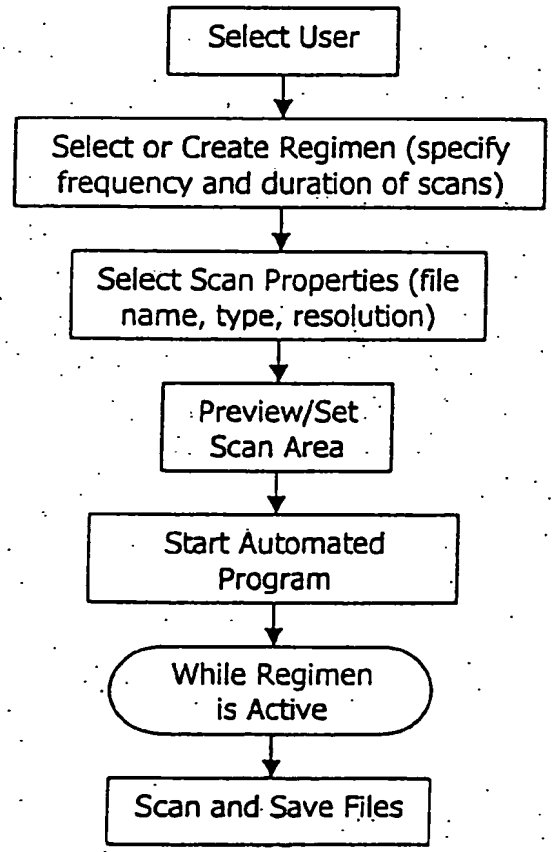



Figure 2: Flowchart of one simple embodiment of the software function

!GUI-Flexcell Scan.vi

Block Diagram

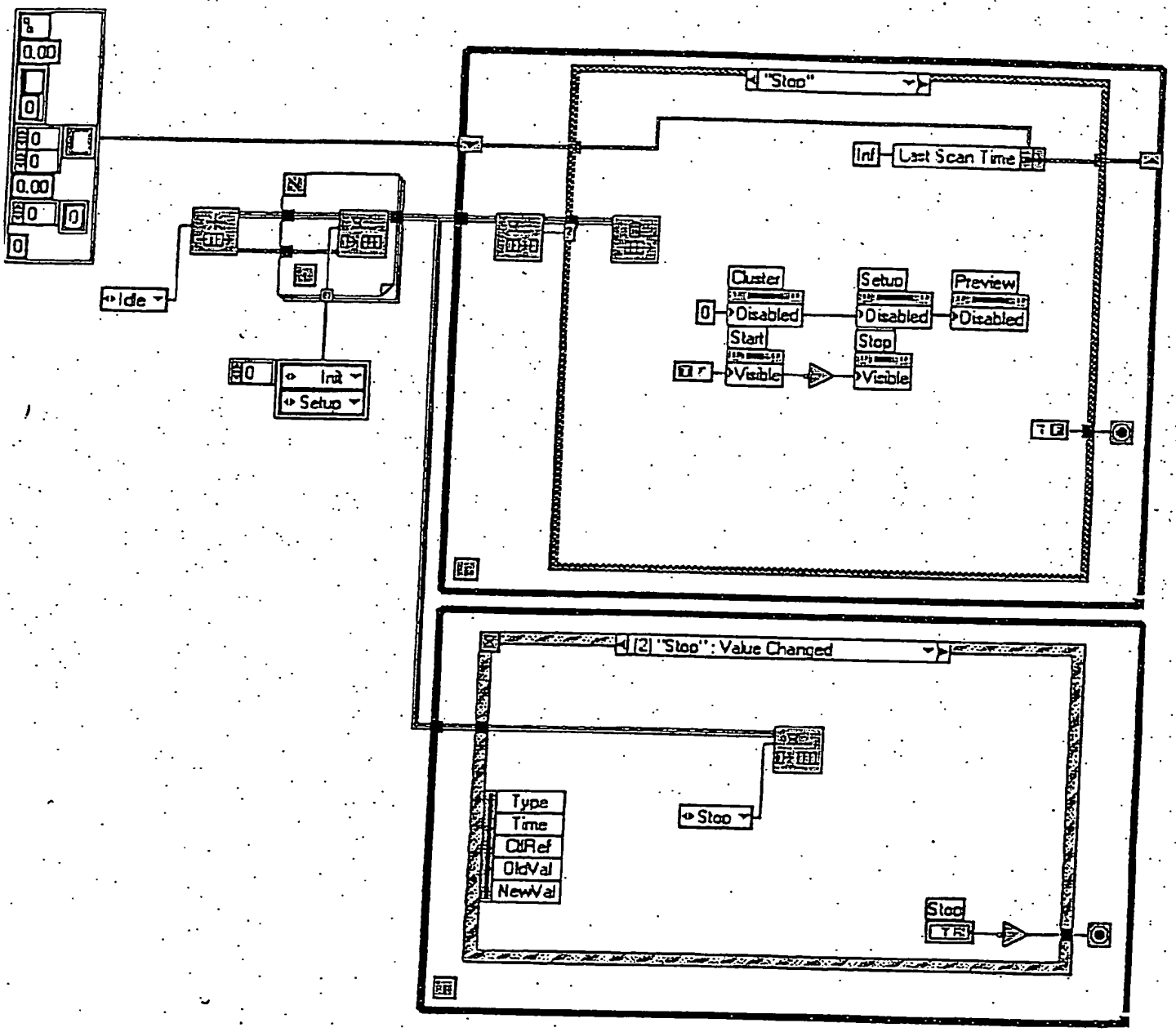



Fig 3

!GUI-Flexcell Scan. vi

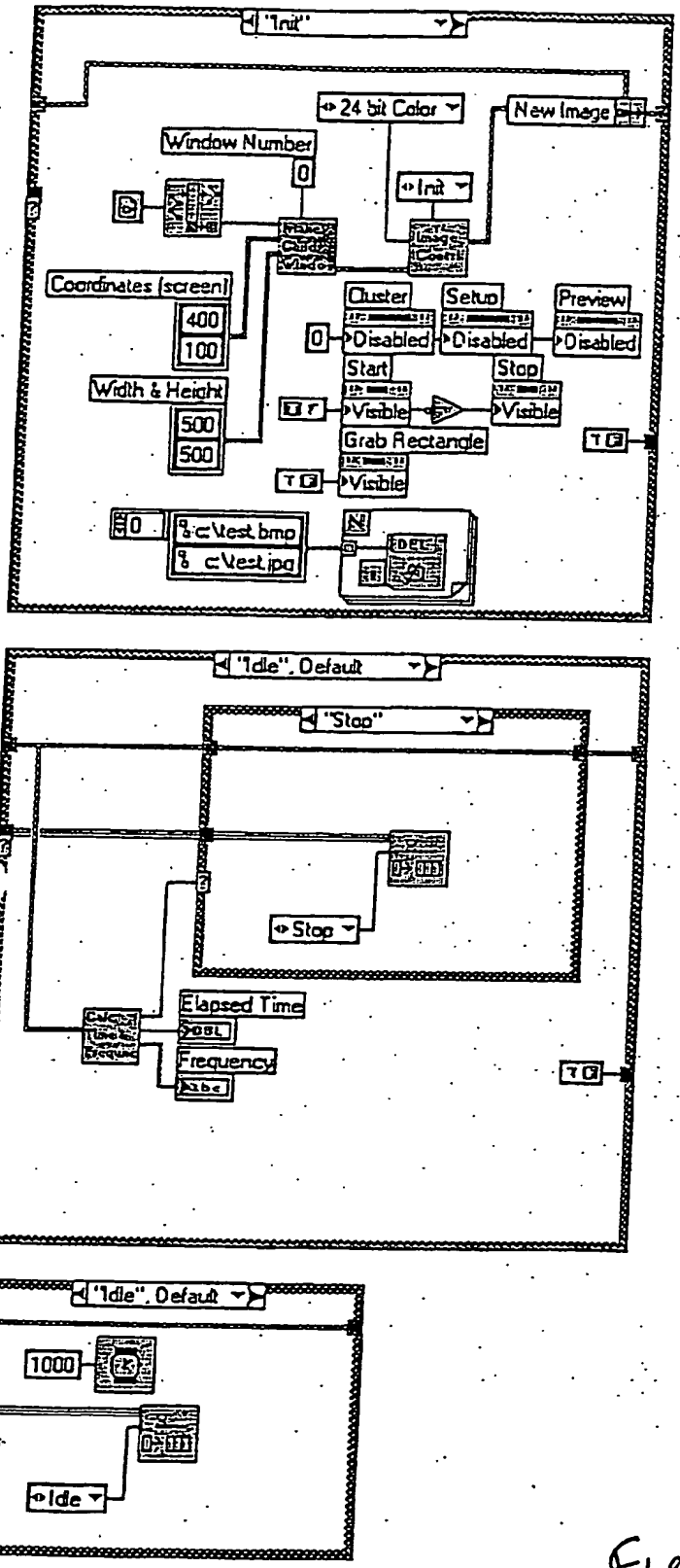



Fig 4

!GUI-Flexcell Scan.vi

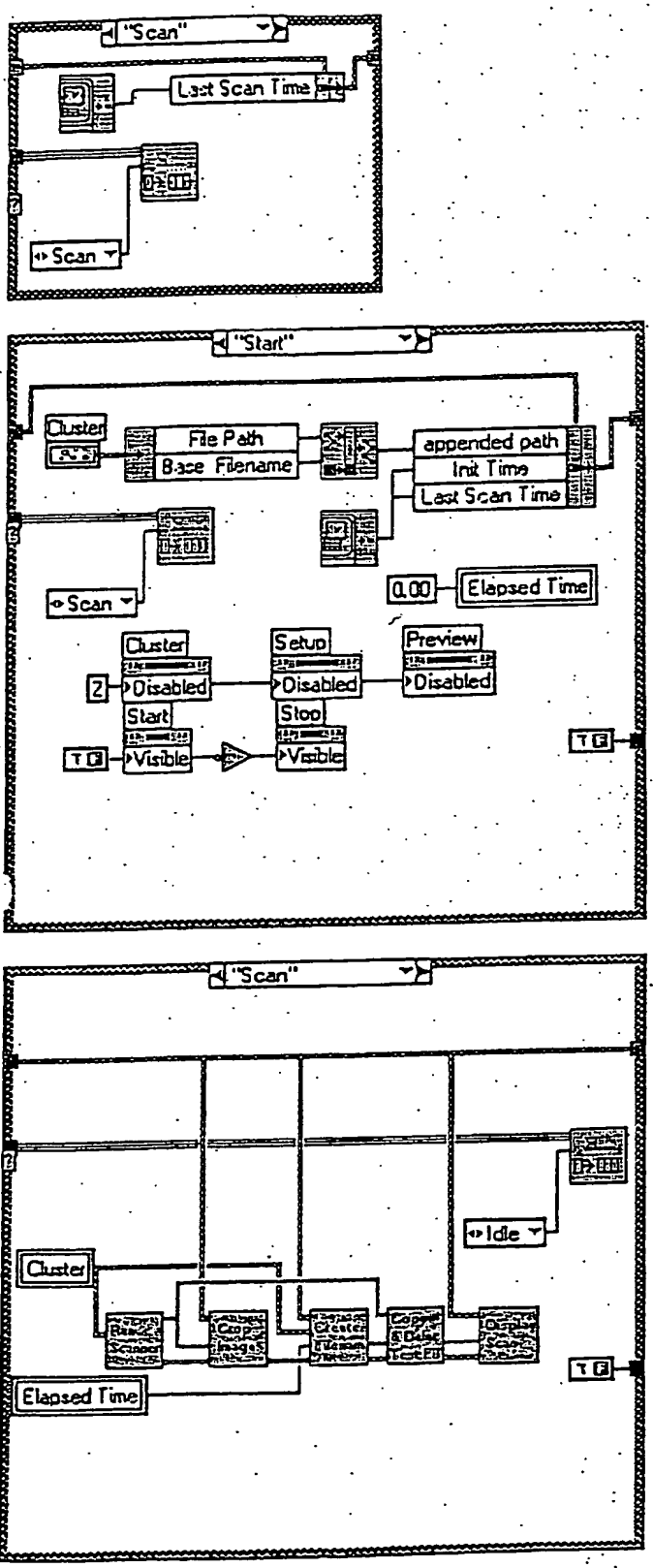



Fig 5

!GUI-Flexcell Scan.vi

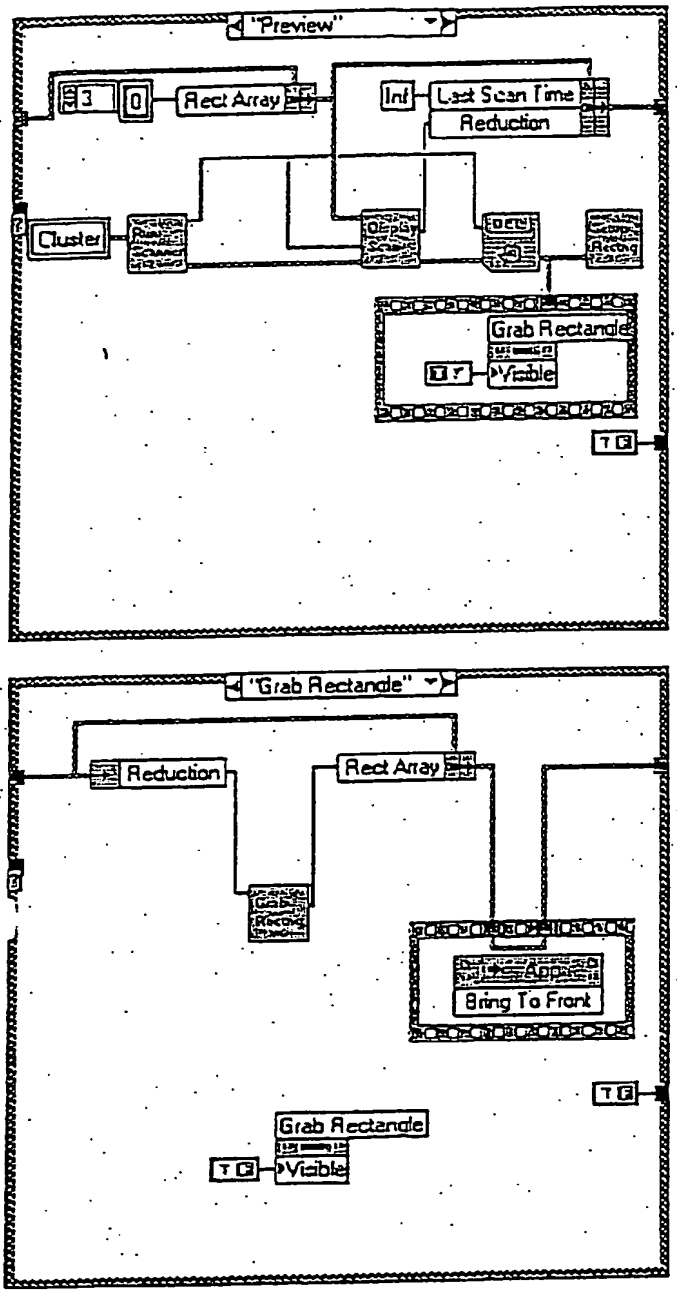
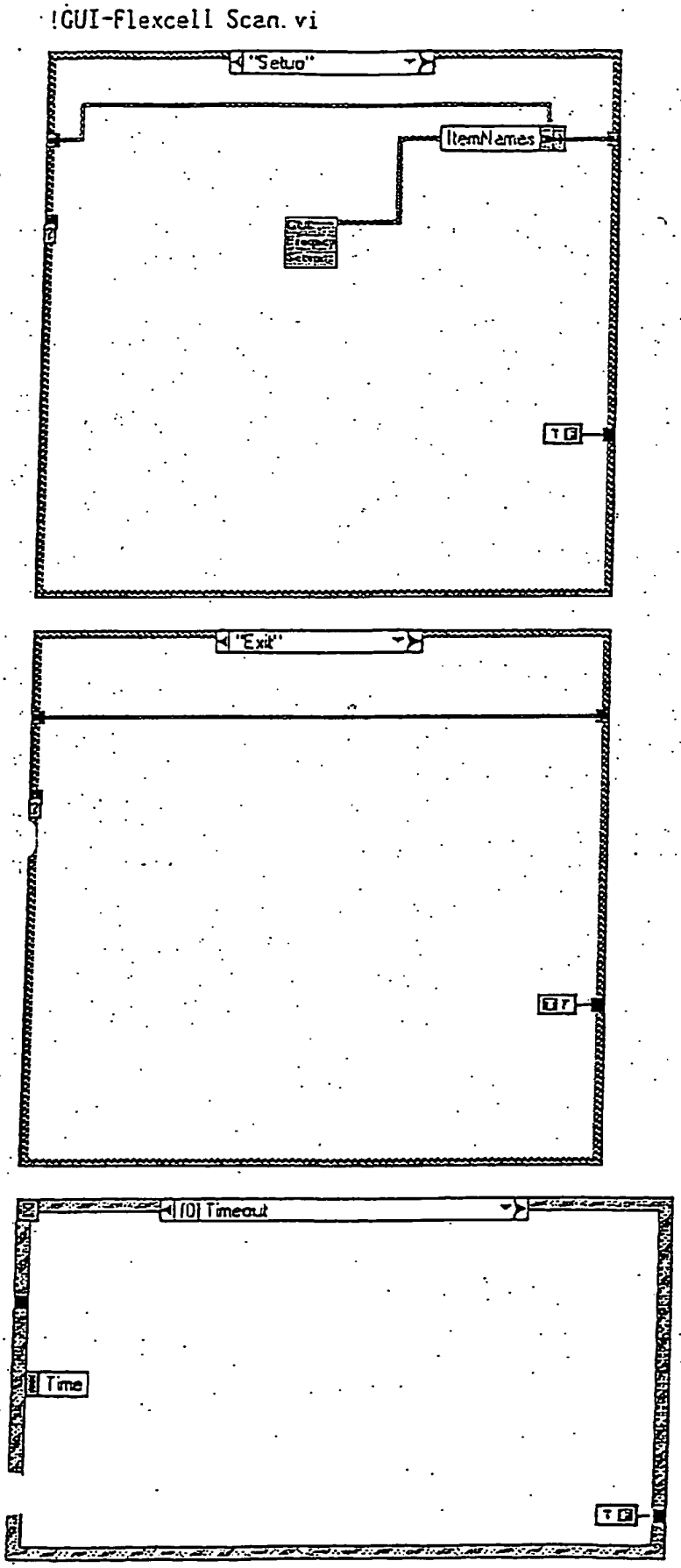



Fig 6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"Setup"
ItemNames
TF
"Exit"
TF
(0) Timeout
Time
TF

Fig 7

!GUI-Flexcell Scan.vi

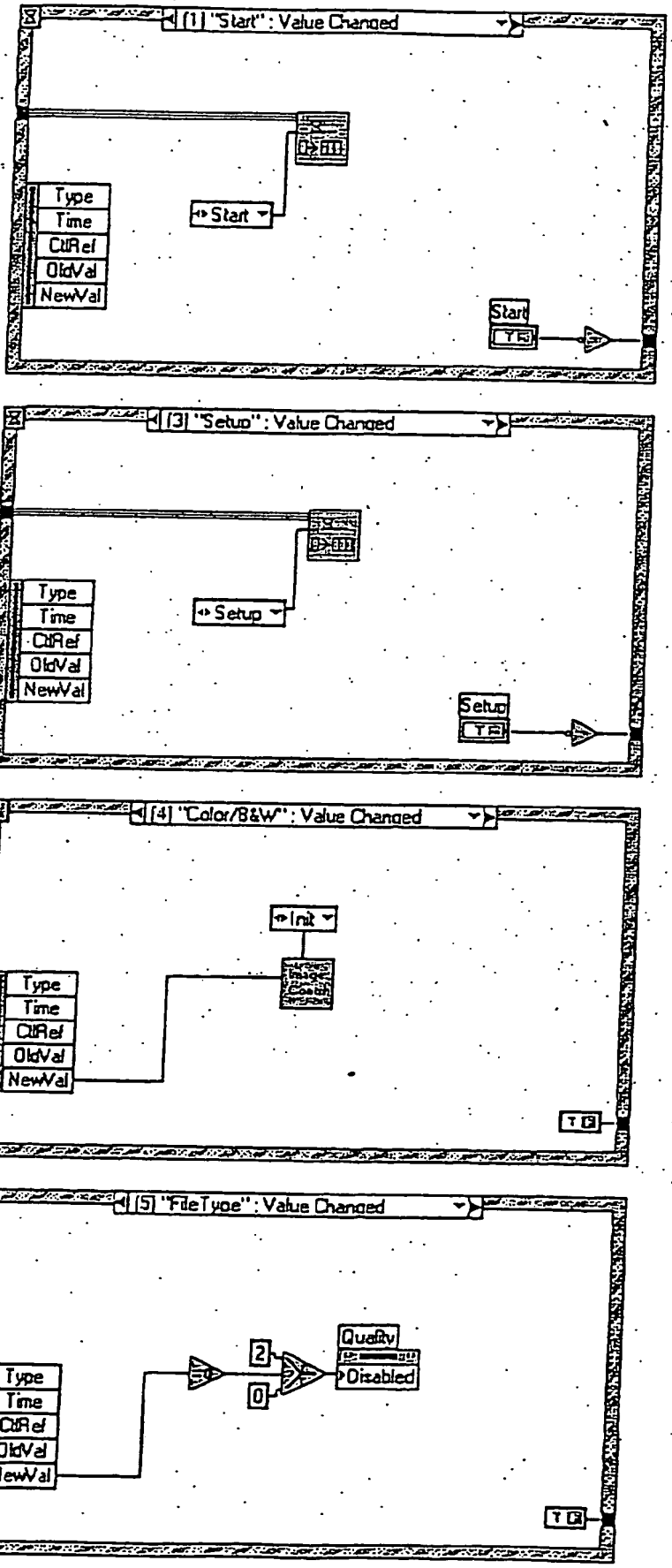



Fig 8

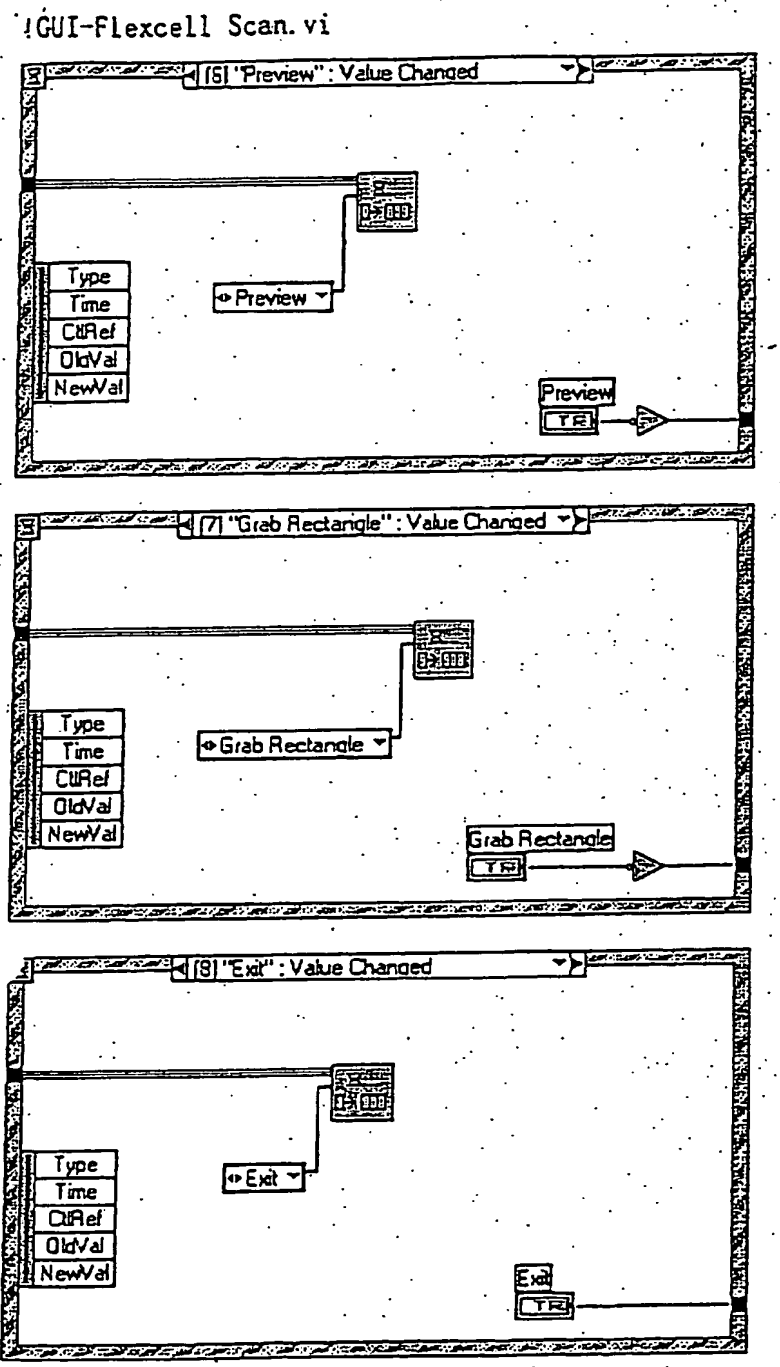

!GUI-Flexcell Scan.vi
[6] "Preview": Value Changed
Type
Time
CtlRef
OldVal
NewVal
Preview
Preview
[7] "Grab Rectangle": Value Changed
Type
Time
CtlRef
OldVal
NewVal
Grab Rectangle
Grab Rectangle
[8] "Exit": Value Changed
Type
Time
CtlRef
OldVal
NewVal
Exit
Exit

Fig 9

GUI-Flexcell Frequency Setup.vi

Block Diagram

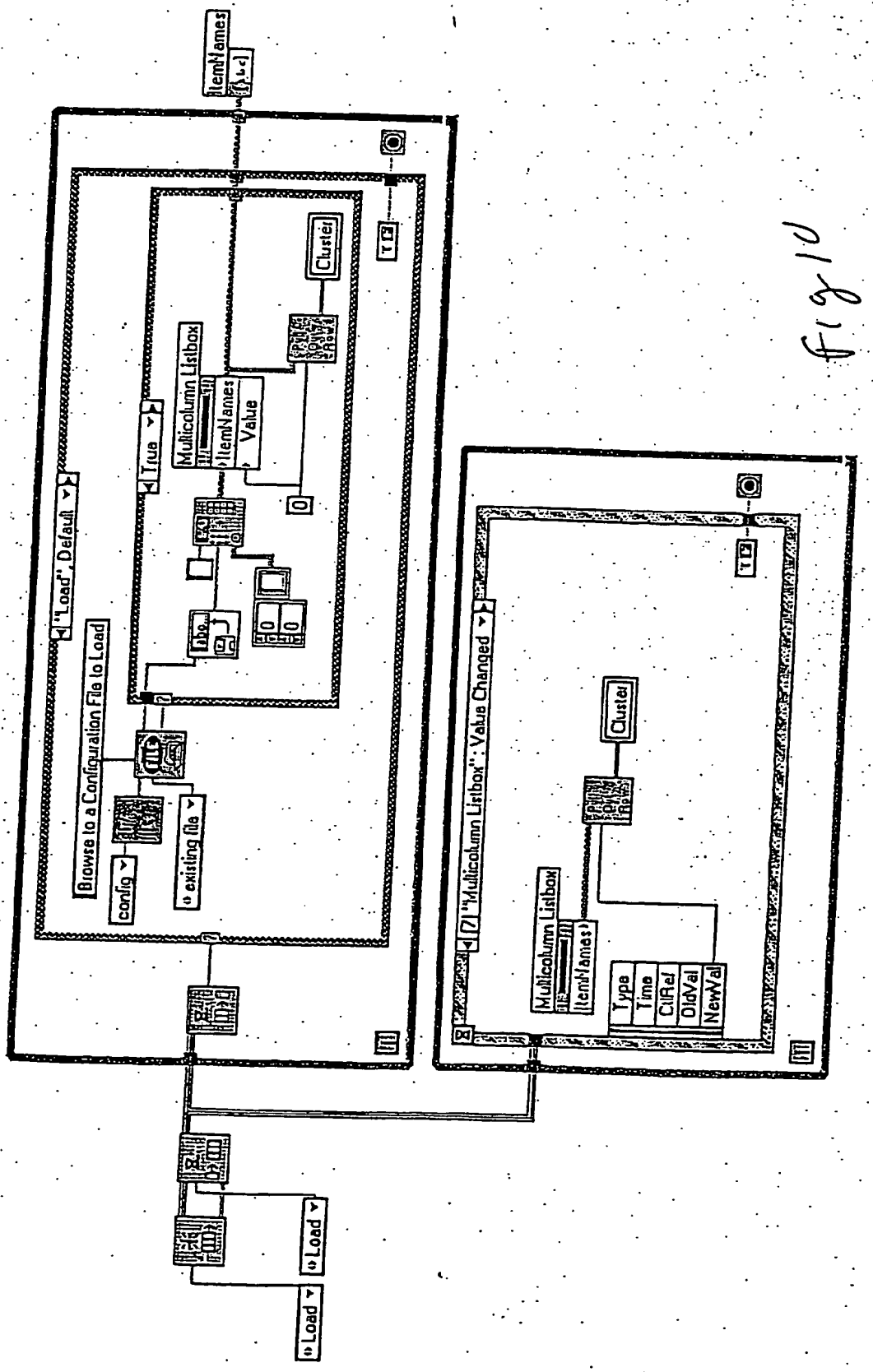
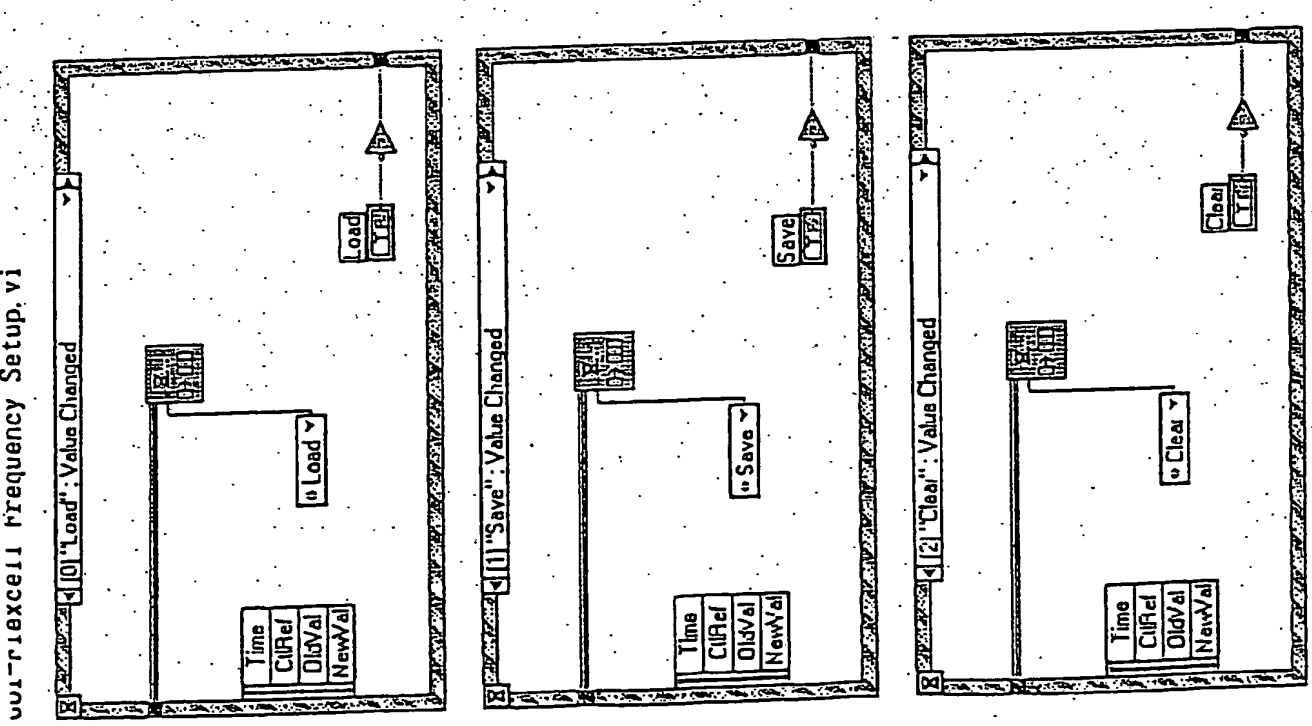



Fig 1C

Fig 11


Load
Load
Time
CtlRef
OldVal
NewVal
[0] "Load": Value Changed
Save
Save
Time
CtlRef
OldVal
NewVal
[1] "Save": Value Changed
Clear
Clear
Time
CtlRef
OldVal
NewVal
[2] "Clear": Value Changed

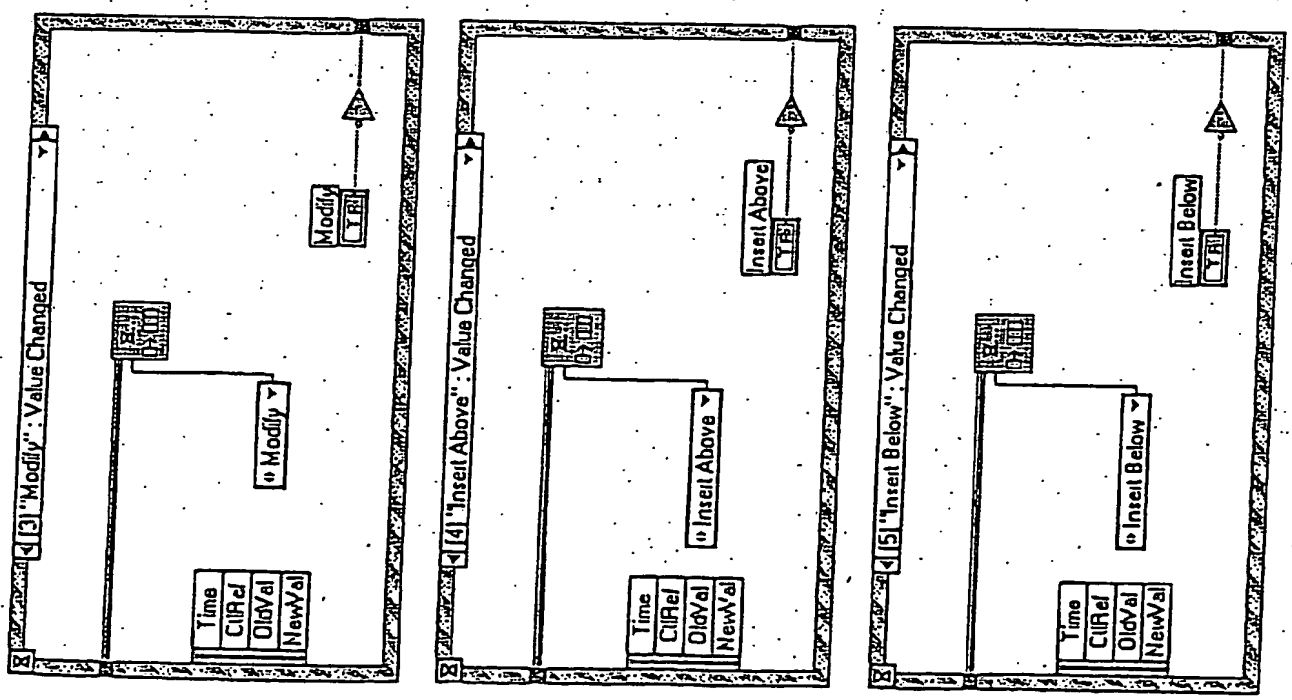
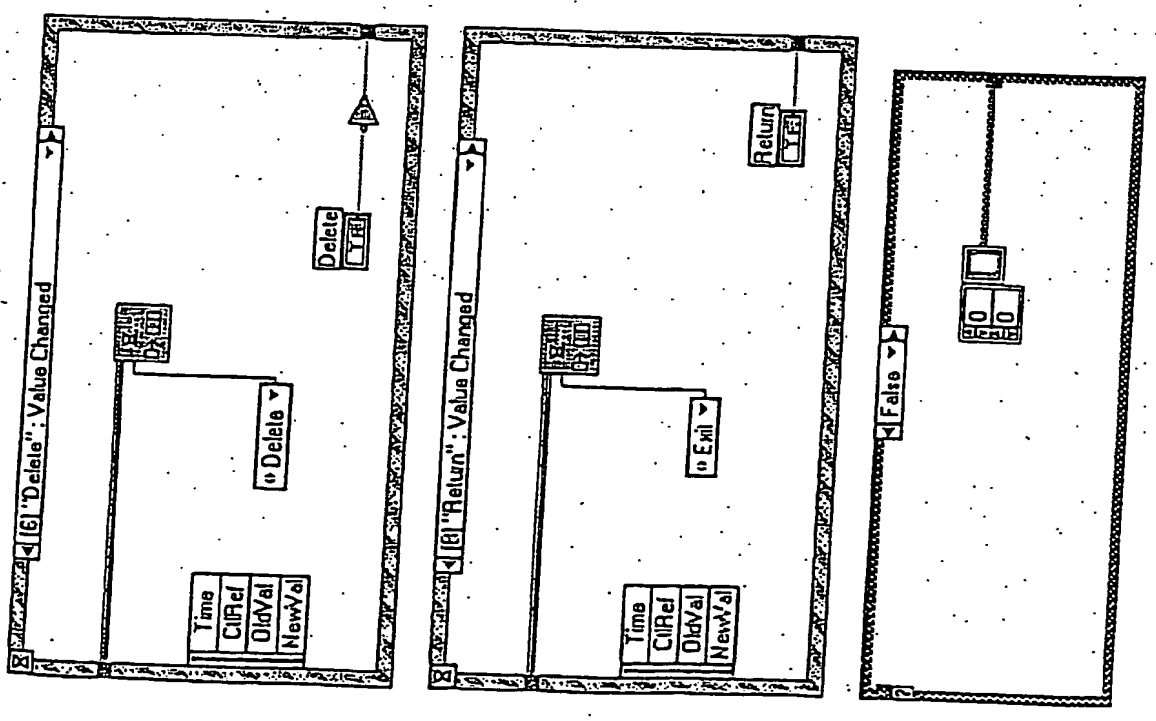

[3] "Modify": Value Changed
Modify
Time
ClkRel
OldVal
NewVal
[4] "Inset Above": Value Changed
Inset Above
Time
ClkRel
OldVal
NewVal
[5] "Inset Below": Value Changed
Inset Below
Time
ClkRel
OldVal
NewVal

Fig 12

Fig 14


Delete
[6] "Delete": Value Changed
Delete
Time
ClkRef
OldVal
NewVal
Return
[6] "Return": Value Changed
Exit
Time
ClkRef
OldVal
NewVal
False

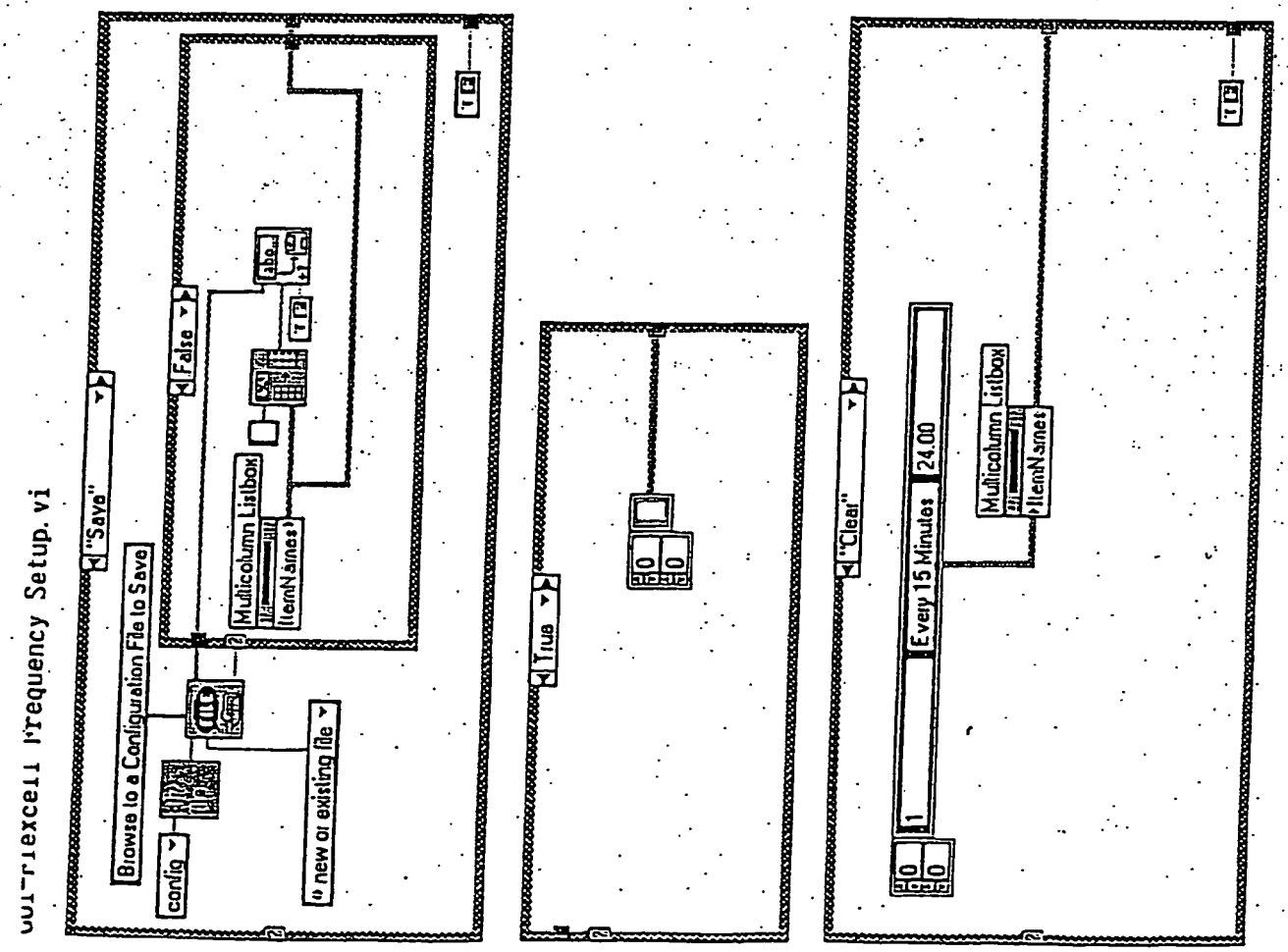

"Save"
Browse to a Configuration File to Save
config
new or existing file
False
Multicolumn Listbox
ItemNames
True
"Clear"
Every 15 Minutes
24.00
Multicolumn Listbox
ItemNames

Fig 14

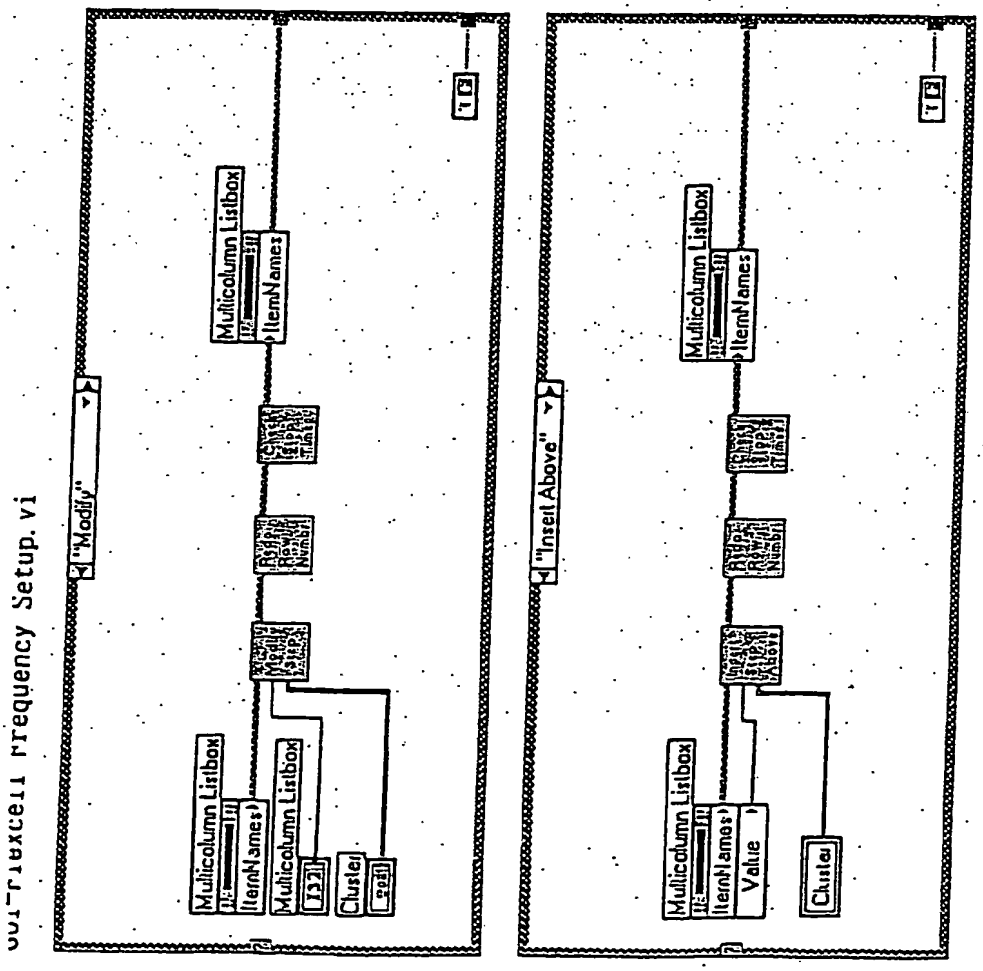
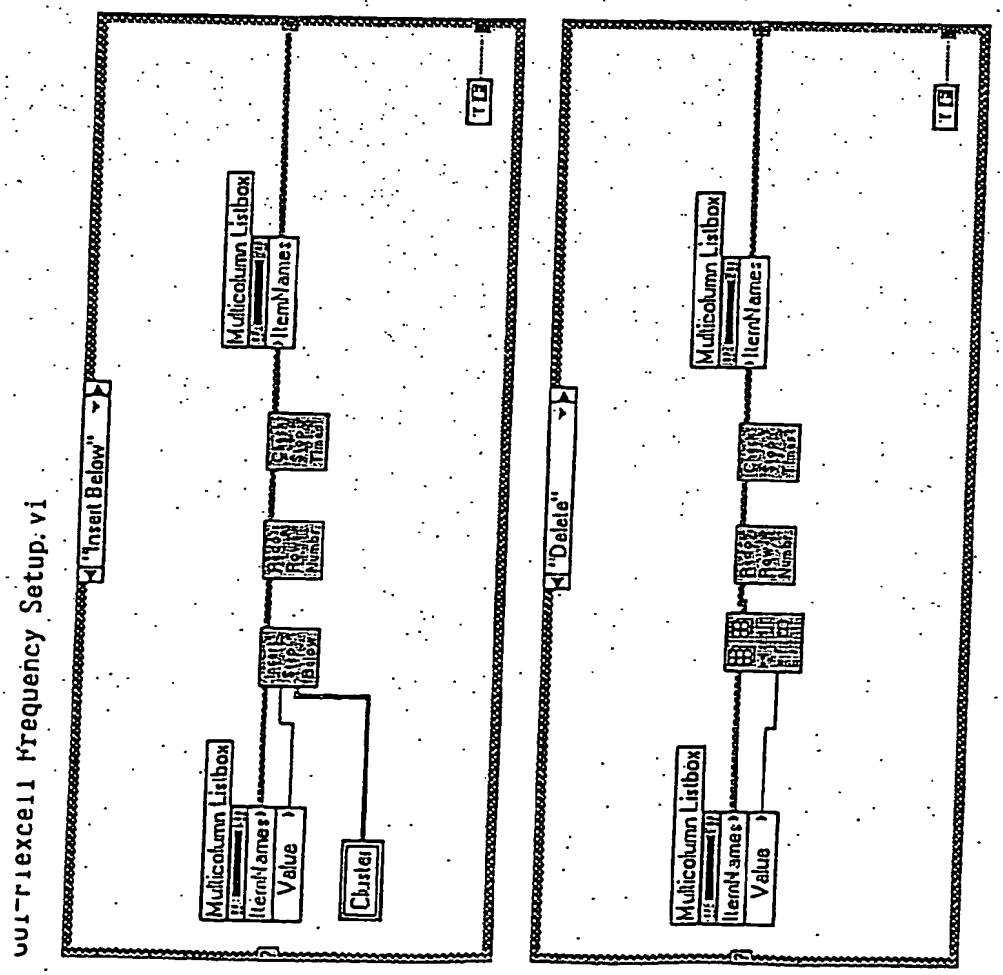

"Modify"
Multicolumn Listbox
ItemNames
Multicolumn Listbox
ItemNames
Multicolumn Listbox
Cluster
"Insert Above"
Multicolumn Listbox
ItemNames
Multicolumn Listbox
ItemNames
Value
Cluster

Fig 15


Multicolumn Listbox
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"Insert Below"
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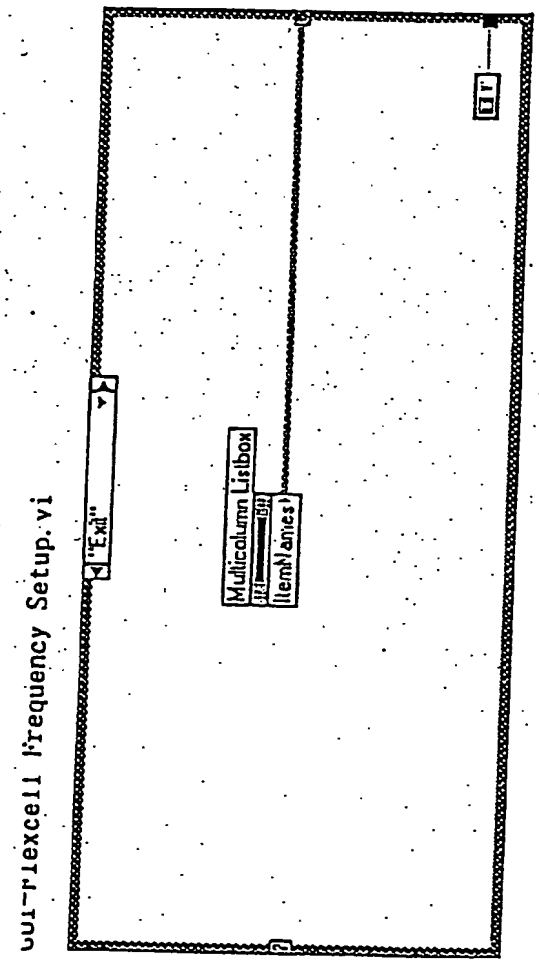
ItemNames
Value
Cluster
TF
"Delete"
Multicolumn Listbox
ItemNames
Value
TF

Fig 16

Fig 17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itemNames
multiexcell Frequency Setup.vi

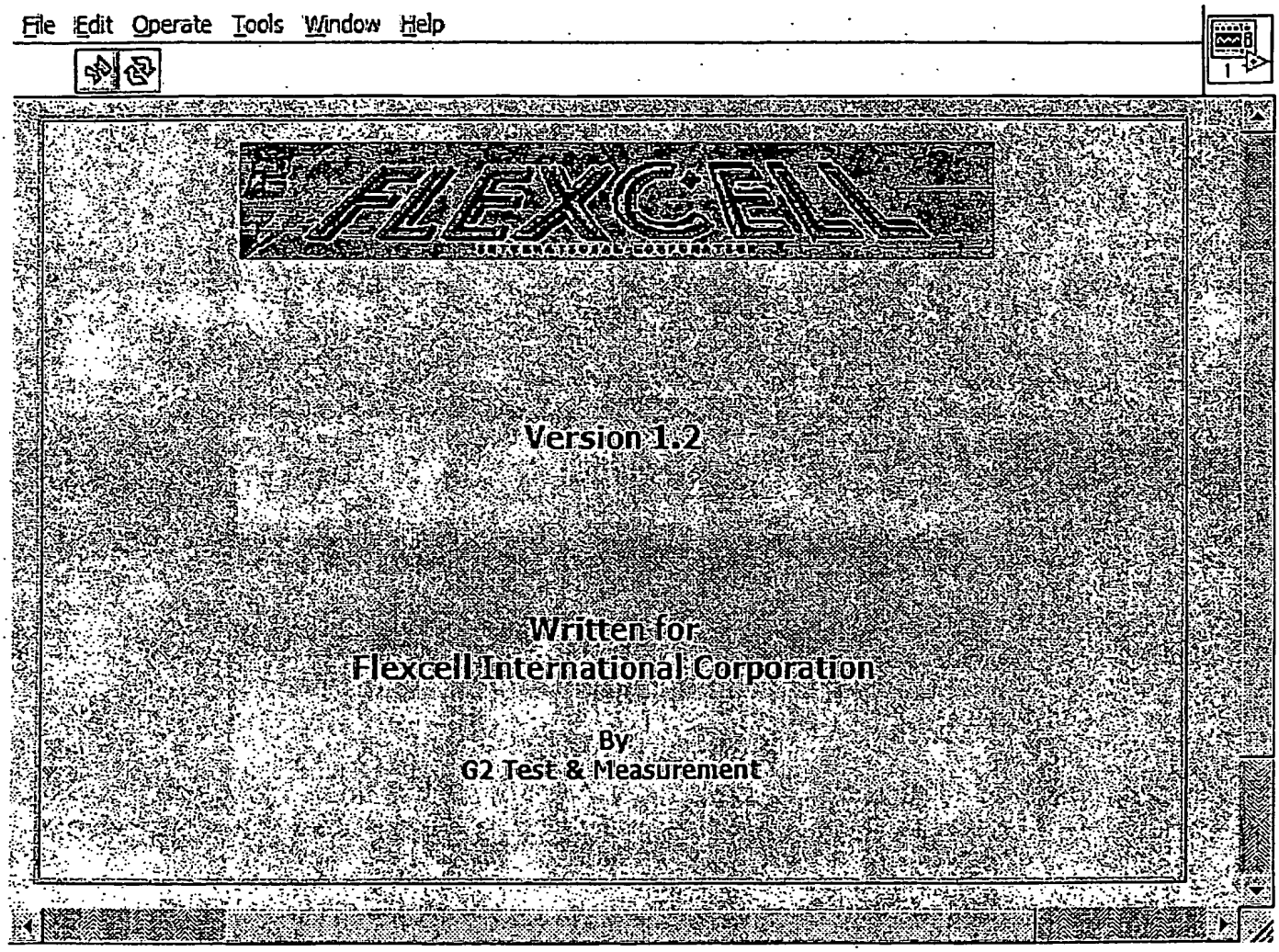

File Edit Operate Tools Window Help
FLEXCELL
Version 1.2
Written for
Flexcell International Corporation
By
G2 Test & Measurement

Fig. 18

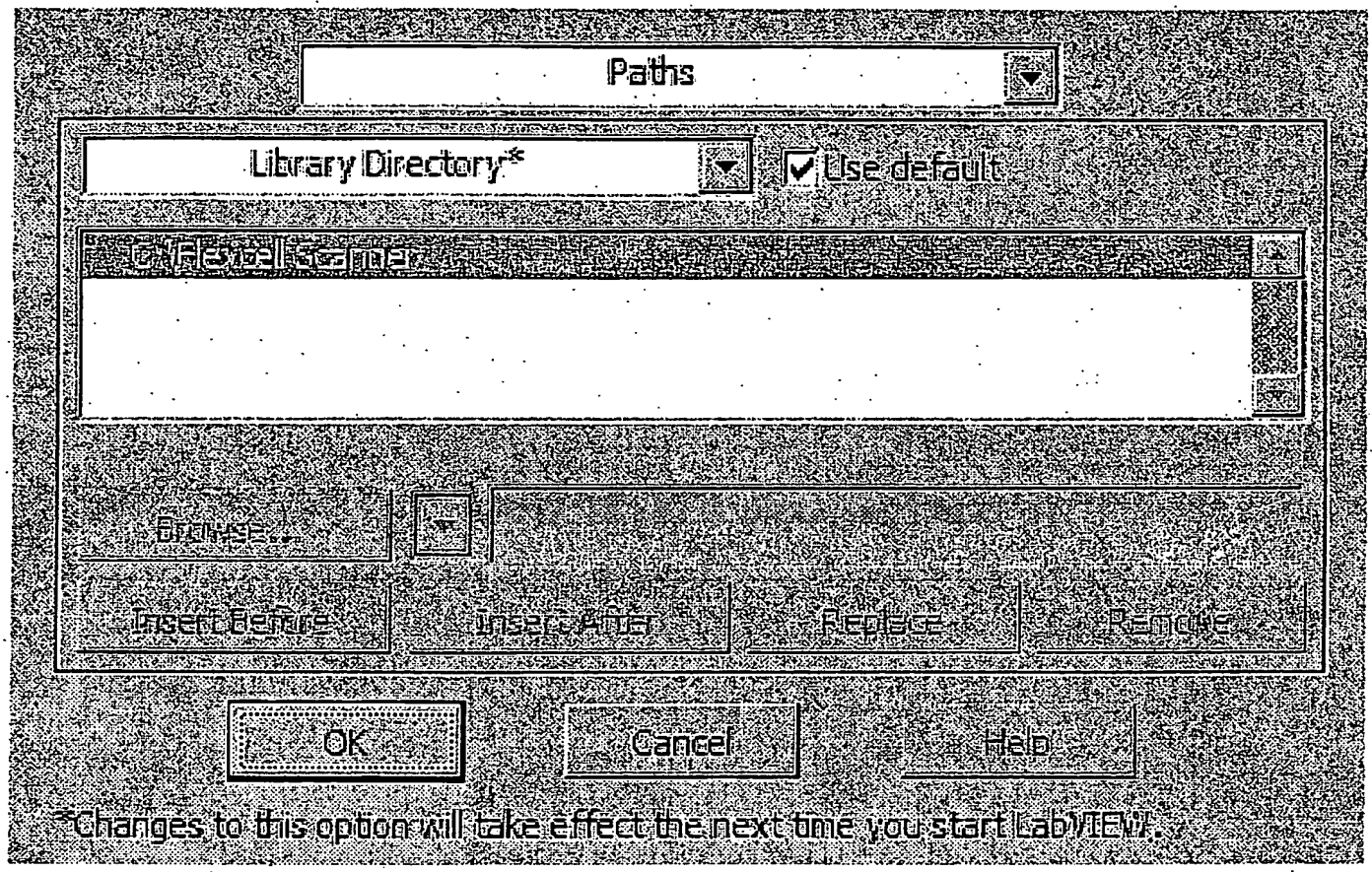
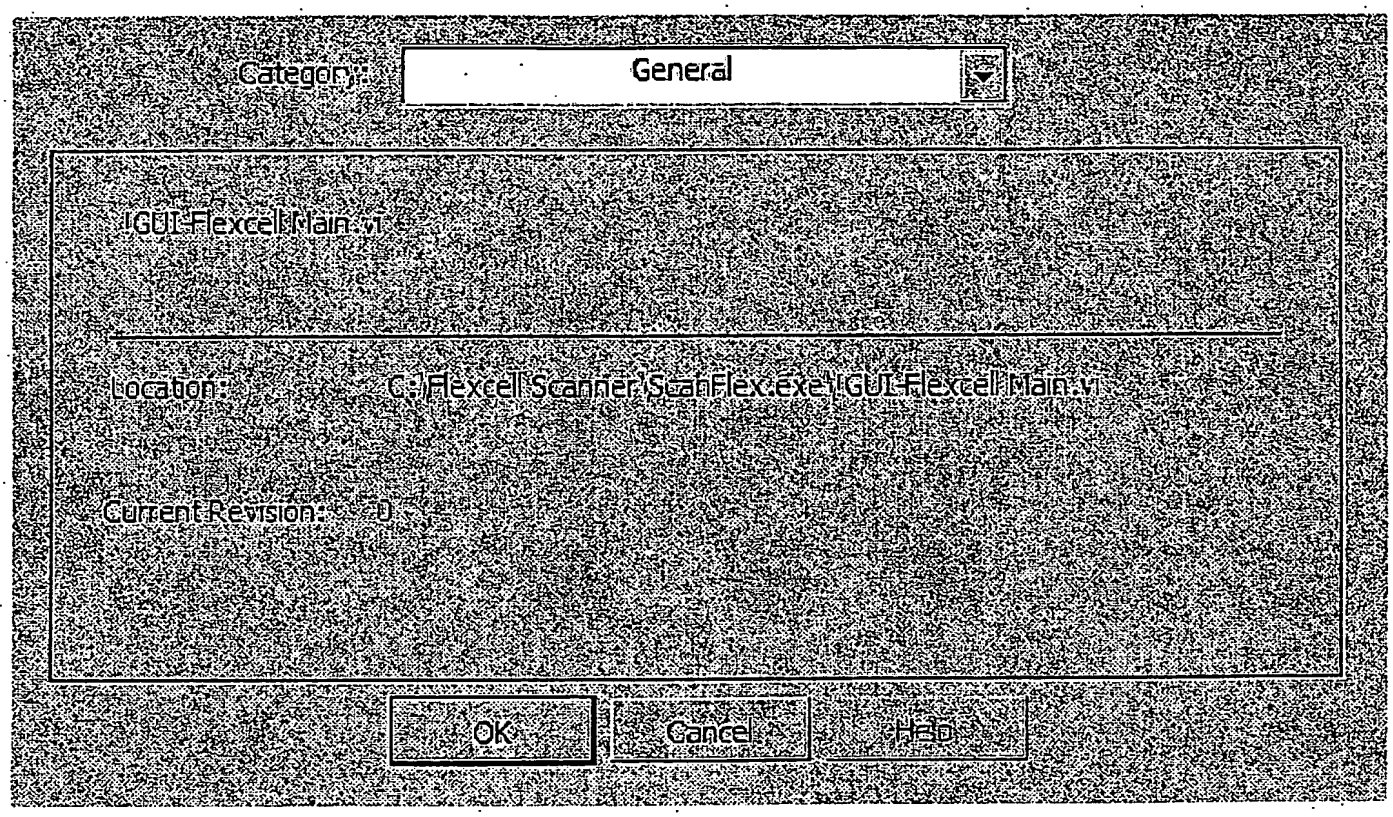

Paths
Library Directory*
Use default
C:\Program Files\LabVIEW
Browse...
Insert Before...
Insert After...
Replace...
Remove
OK
Cancel
Help
*Changes to this option will take effect the next time you start LabVIEW.

Fig. 19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General
GUI Flexcel Main.vi
Location:
C:\Flexcel Scanner\ScanFlex.exe\GUI Flexcel Main.vi
Current Revision:
0
OK
Cancel
Help

Fig. 20

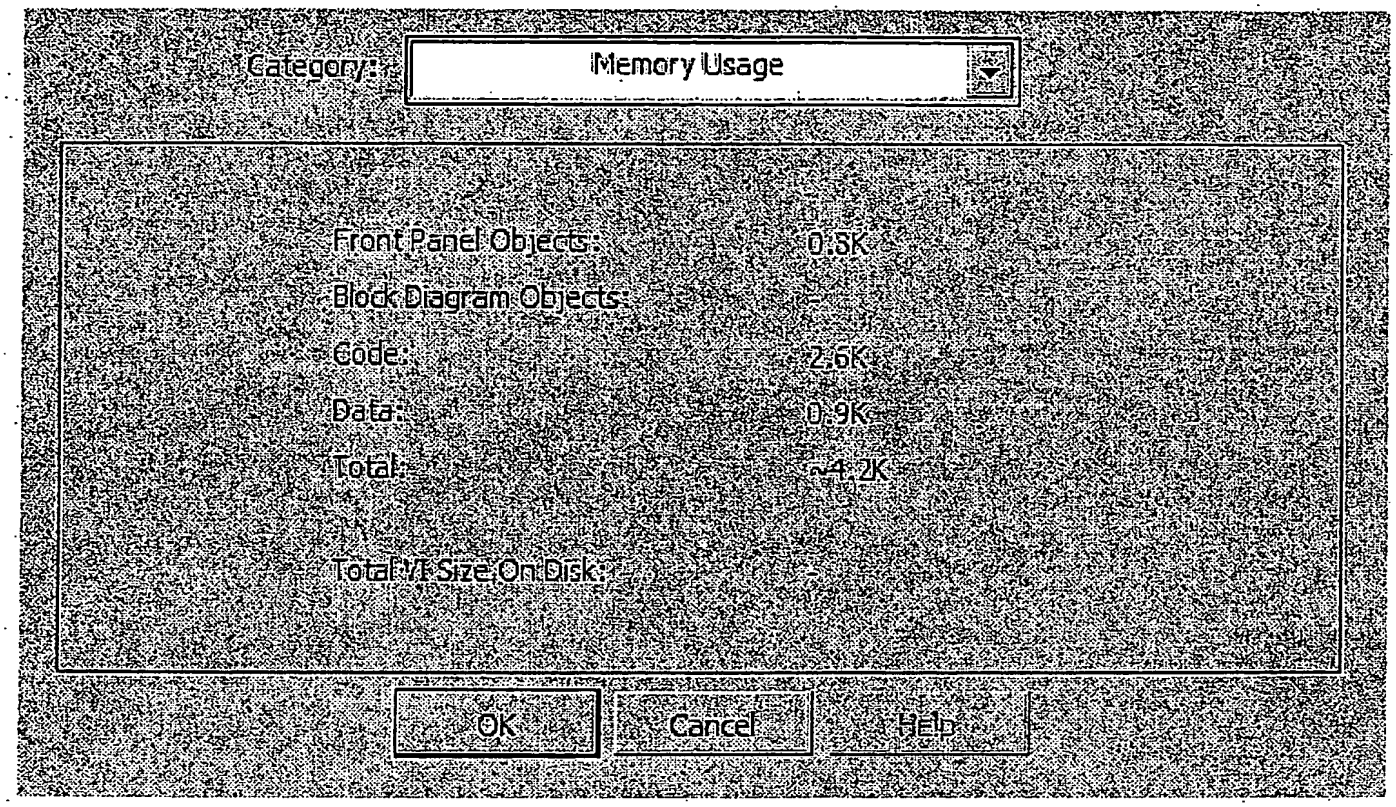
Category:
Memory Usage
Front Panel Objects:
0.8K
Block Diagram Objects:
Code:
2.6K
Data:
0.9K
Total:
4.2K
Total VI Size On Disk:
OK
Cancel
Help

Fig. 21

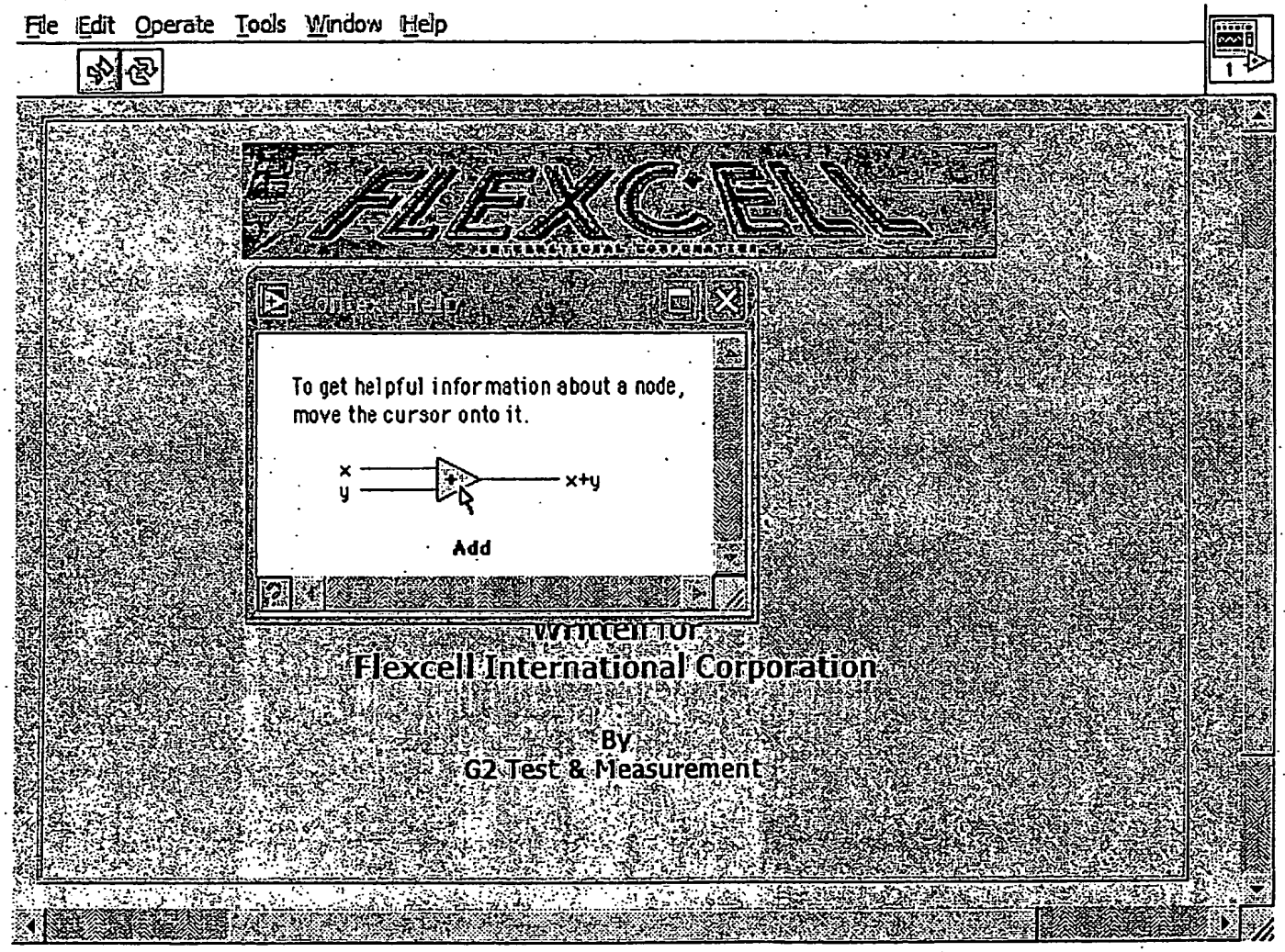
File Edit Operate Tools Window Help
FLEXCELL
To get helpful information about a node, move the cursor onto it.
x
y
x+y
Add
Written for
Flexcell International Corporation
By
G2 Test & Measurement

Fig. 22

Patent Information

For the most current list of patents covering this product, please refer to ni.com/legal/patents.
The LabVIEW software is covered by one or more of the following Patents:
United States Patent No(s): 4,901,221; 4,914,568; 5,291,587; 5,301,301; 5,301,336; 5,475,851; 5,481,740; 5,481,741; 5,497,500; 5,504,917; 5,533,983; 5,610,828; 5,652,909; 5,732,277; 5,734,863; 5,737,622; 5,764,546; 5,784,275; 5,821,934; 5,847,953; 5,905,649; 5,920,479; 5,974,254; 5,990,906; 6,064,812; 6,064,816; 6,102,965; 6,138,270; D384051; D387750; D384050; D384052
European Patent No(s).: 0242131
Japanese Patent No(s).: 3,016,783
Canadian Patent No(s).: 1285655

Various other software products may be included with this version of LabVIEW. If any software products listed below are included, they are covered by various Patents as follows:

The LabVIEW Signal Processing Toolset is covered by one or more of the following Patents:
U.S. Patent No(s).: 5,353,233; 6,108,609
European Patent No(s).: 0632899
Japanese Patent No(s).: 2,697,957

The LabVIEW Datalogging and Supervisory Control Module is covered by one or more of the following Patents:
U.S. Patent No(s).: 5,966,532; 6,053,951

LabVIEW Real Time is covered by one or more of the following Patents:
U.S. Patent No(s).: 6,173,438

The LabVIEW PID Control Toolset is covered by one or more of the following Patents:
U.S. Patent No(s).: 6,081,751

The IVI Driver Toolset is covered by one or more of the following Patents:
U.S. Patent No(s).: 5,963,726; 6,085,156

The NI-VISA software is covered by one or more of the following Patents:
U.S. Patent No(s).: 5,724,272; 5,710,727; 5,847,955; 5,640,572; 5,771,388; 5,627,988; 5,717,614

The NI-DAQ software is covered by one or more of the following Patents:
U.S. Patent No(s).: 5,619,702; 6,067,584; 6,096,094; 6,052,743; 6,148,438; 5,926,775; 5,987,530; 6,073,205

The NI-488 or NI-488.2 (NI-GPIB) software is covered by one or more of the following Patents:
U.S. Patent No(s).: 5,974,541; 5,964,892; 5,958,028; 5,987,530; 6,073,205

The NI-FBUS software, including one or more of the NI-FBUS Configurator software or the NI-FBUS Monitor software, is covered by one or more of the following Patents:
U.S. Patent No(s).: 5,854,890; 5,796,721; 5,850,523; 5,971,581; 6,141,596; 6,076,952; 5,978,850;

Fig. 23

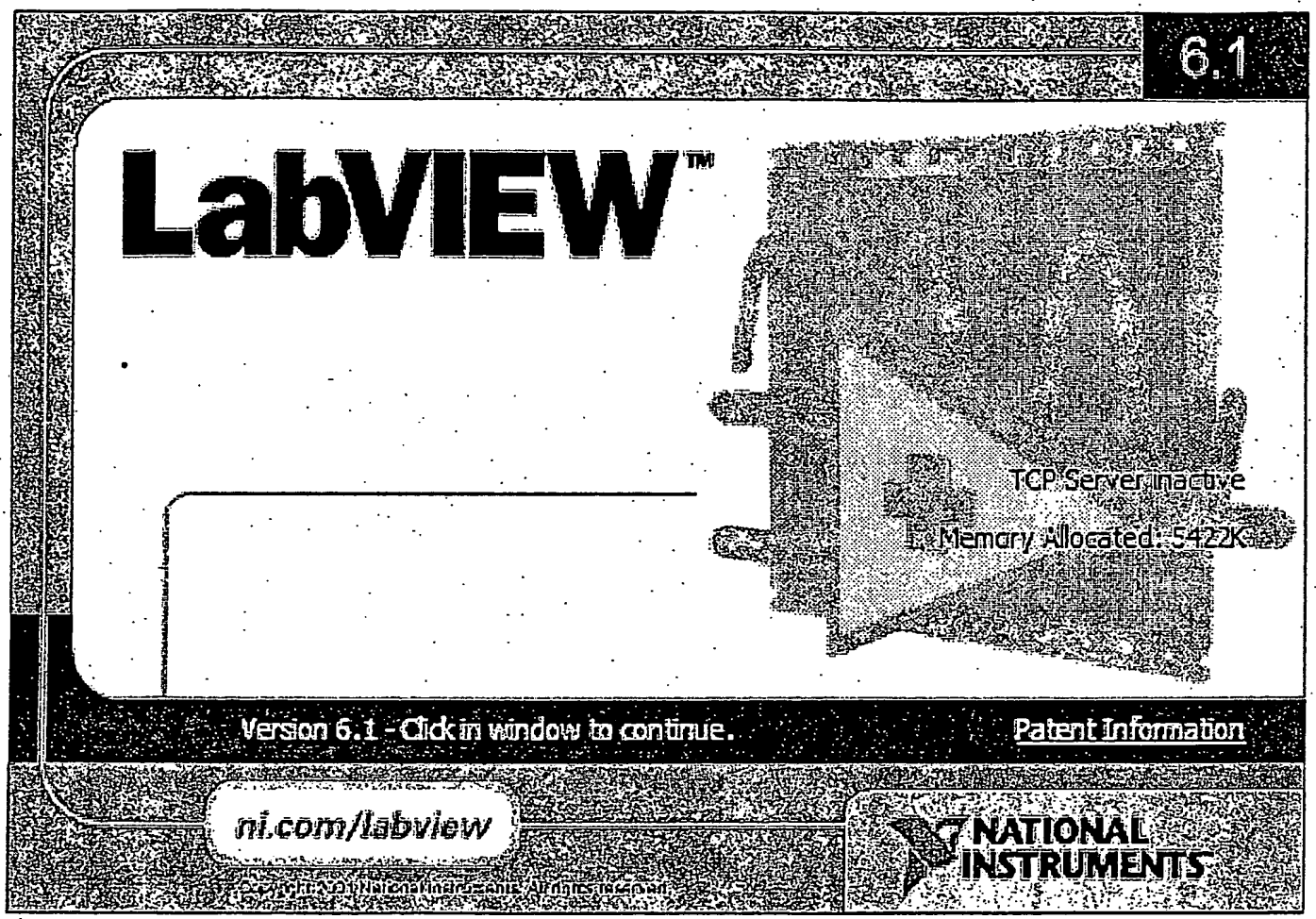
6.1
LabVIEW™
TCP Server inactive
Memory Allocated: 5422K
Version 6.1 - Click in window to continue.
Patent Information
ni.com/labview
NATIONAL INSTRUMENTS

Fig. 21

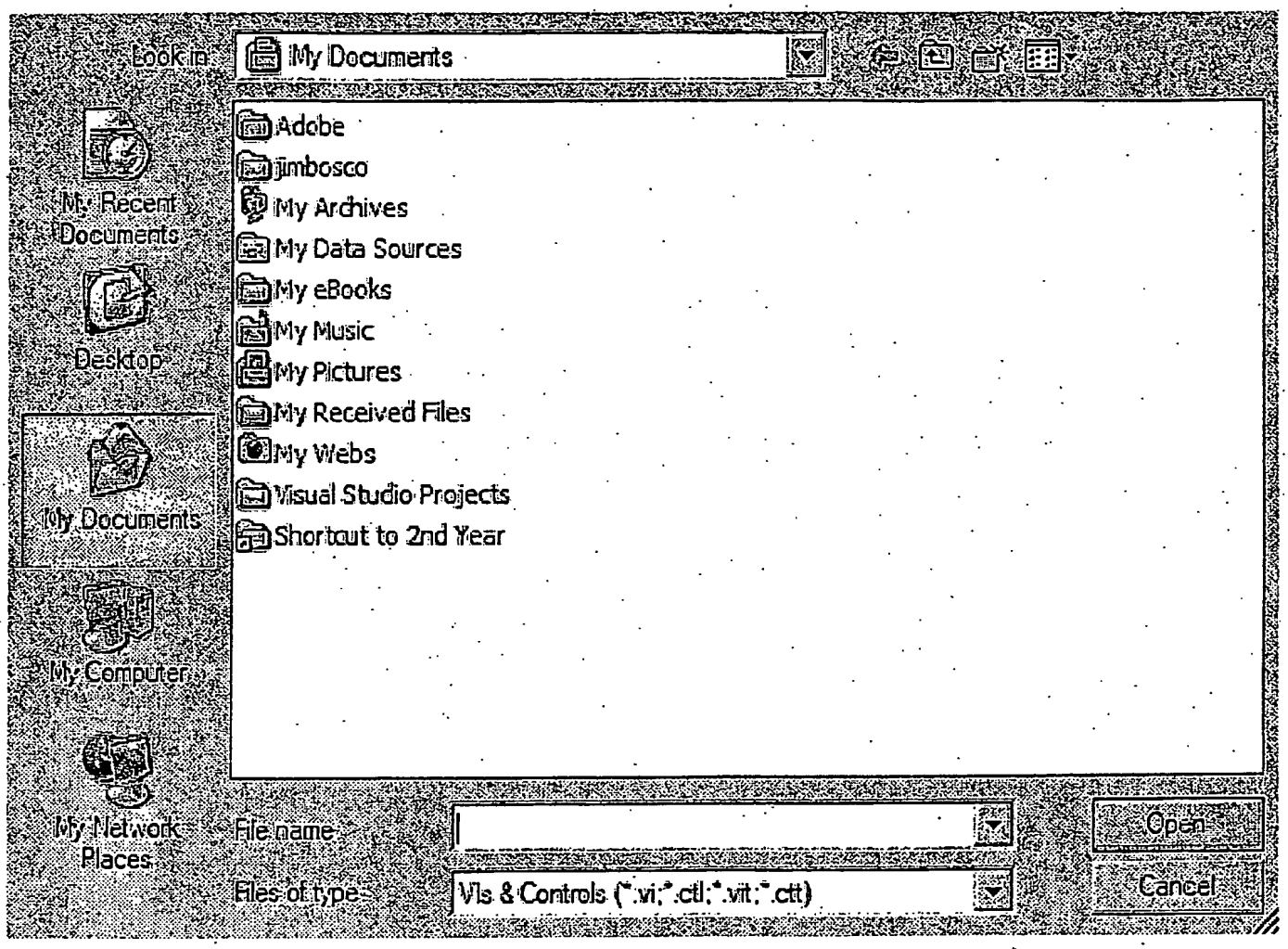
Look in:
My Documents
Adobe
jimbosco
My Archives
My Data Sources
My eBooks
My Music
My Pictures
My Received Files
My Webs
Visual Studio Projects
Shortcut to 2nd Year
My Recent Documents
Desktop
My Documents
My Computer
My Network Places
File name:
Files of type:
Vis & Controls (*.vi;*.ctl;*.vit;*.ctt)
Open
Cancel

Fig. 25

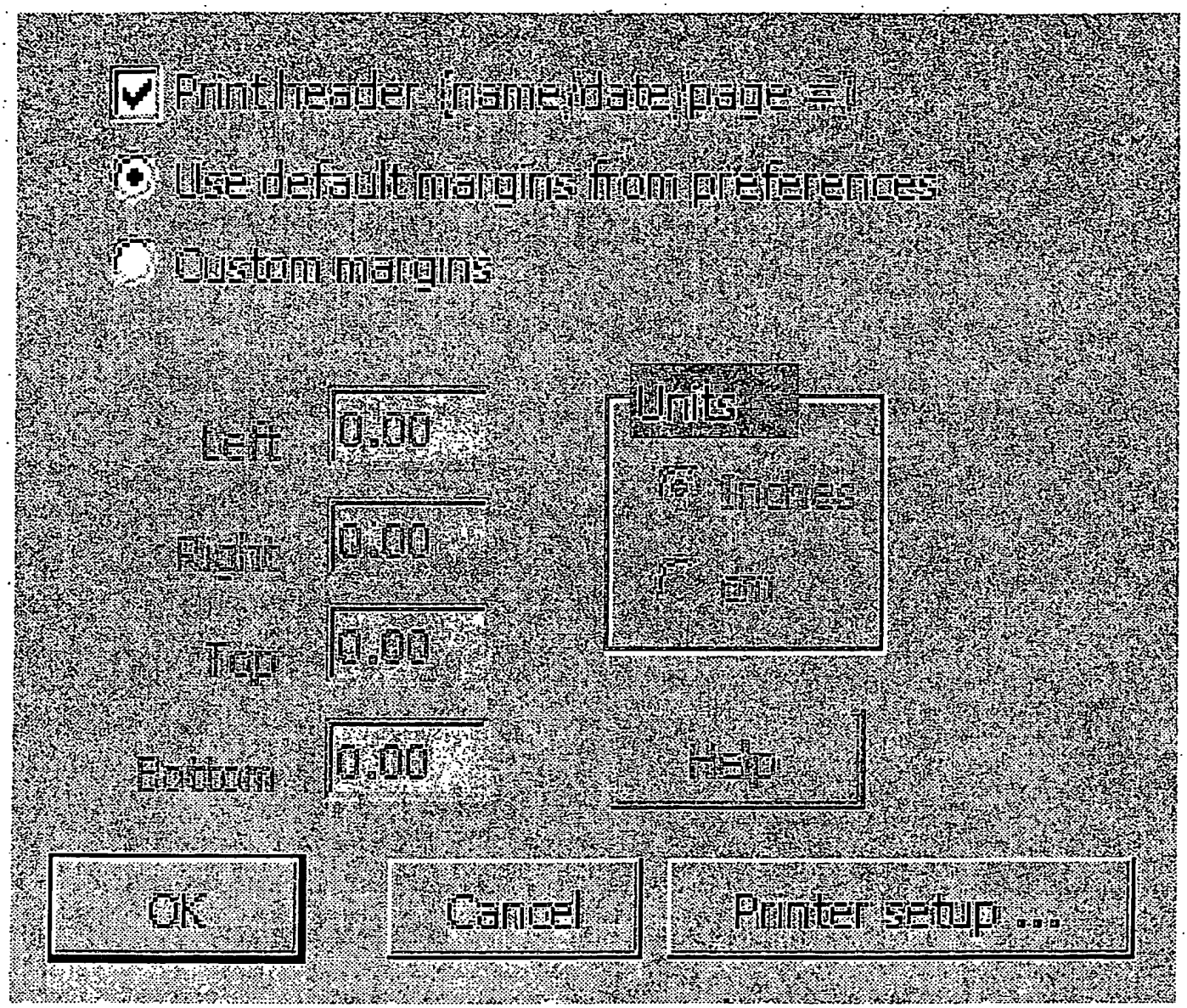
Print header: name, date, page #
Use default margins from preferences
Custom margins
Left
0.00
Right
0.00
Top
0.00
Bottom
0.00
Units
Inches
cm
Pts
OK
Cancel
Printer setup...

Fig. 26

Acquire.exe is a Windows utility that transfers images from TWAIN devices to files in BMP or JPEG format. Because it is controlled by command-line parameters, Acquire.exe is suitable for use from the DOS box, as a spawned sub-program, or from a desktop icon.

About

Usage: ACQUIRE [options] [filename]

The options are described in the table below. Case is not important. /INCHES and /inches are equivalent. If no options are given, /H/NOSCAN is assumed. filename is the file name or full path of the saved image file. If no extension is given, .bmp or .jpg is added. If no filename is given, the user is prompted for it.

| Option | Definition | Example |
|---|---|---|
| /AUTOBRIGHTn | Set automatic brightness 1=On, 0=Off | /autobright1 |
| /ADF | Enable automatic feeding of documents | /adf |
| /Bn | Set Brightness to n. (very device specific) | /b500 |
| /BMP | Write .bmp files (default) | /bmp |
| /BW | Request 1-bit black & white | /BW |
| /cm | Set units to centimeters | /cm |
| /Cn | Set Contrast to n. (very device specific) | /C0 |
| /DUP | Use duplex scanning | /dup |
| /FEEDER | Select document feeder | /Feeder |
| /GAMMAn | Set gamma to n | /Gamma1.8 |
| /GRAY | Request 8-bit grayscale | /Gray |
| /H or /? | Display this help text (and exit) | /? |
| /HIDE | Ask the imaging device to hide its dialog | /hide |
| /HIGHn | Set highlight value = n | /high248 |
| /Hn | Set Height to scan (in units) | /H10 |
| /I or /inches | Set units to inches (default) | /i |
| /JPEG | Write .jpg files (JPEG/JFIF format) with quality 75 ("good") | /jpeg |
| /JPEGn | Write .jpg files with quality n (1..100) | /jpeg50 |
| /LOG | Log internal TWAIN activity to c:\eztwain.log | /log |
| /N or /Nn | Scan multiple images (up to n) | /n /n3 |
| note: | Multiple images are saved to filename1, filename2, ... | |
| /NOINDIC | Request no progress indicators | /noindic |
| /NOSCAN | Don't scan | /noScan |
| /NOUI | Request no scan dialog (Same as /HIDE above) | /noUI |
| /PAPER=s | Select paper size: A3,A4,A5,B4,B5,B6,LETTER,LEGAL | /paper=B4 |
| /PAPER=x | Select paper type n (see TWSS_* in TWAIN.H) | /Paper=3 (letter) |
| /PIXELS | Set units to pixels | /pixels |
| /REFL | Set light path to Reflective | /refl |
| /RGB | Request 24-bit color | /rgb |
| /Rn or /R=n | Set Resolution to n in pixels per unit | /r200 |
| /SCAN | Scan an image, even if other options indicate not | /scan |
| /SHADOWn | Set shadow value = n | /shadow10 |
| /S=s | Use source whose name contains the string s | /s=Fujitsu |
| /SS | Display the TWAIN Select Source dialog (and exit) | /SS |
| /Tn | Set Threshold to n | /T128 |
| /TRANS | Set light path to Transmissive | /trans |
| /V | Display version information (and exit) | /v |
| /Wn | Set Width to scan (in units) | /W6.2 |
| /Xn | Set left edge of the scan area (units from left) | /X1.5 |
| /Yn | Set top edge of the scan area (units from top) | /Y7.25 |

Fig. 27

# MATERIALS and METHODS

Human tendon internal fibroblasts (HTIF, $2x10^5$ cells/100 $\mu$l/ specimen) were plated in linear, tethered, collagen gels in TissueTrain™ culture plates (Figure 1).

After 2 hours, when the gels had solidified, the culture plates were removed from the FX-4000TT Tissue Train™ Culture System and placed on the glass of a Plustek OpticPro U24 flatbed scanner, in the incubator (Figure 2).

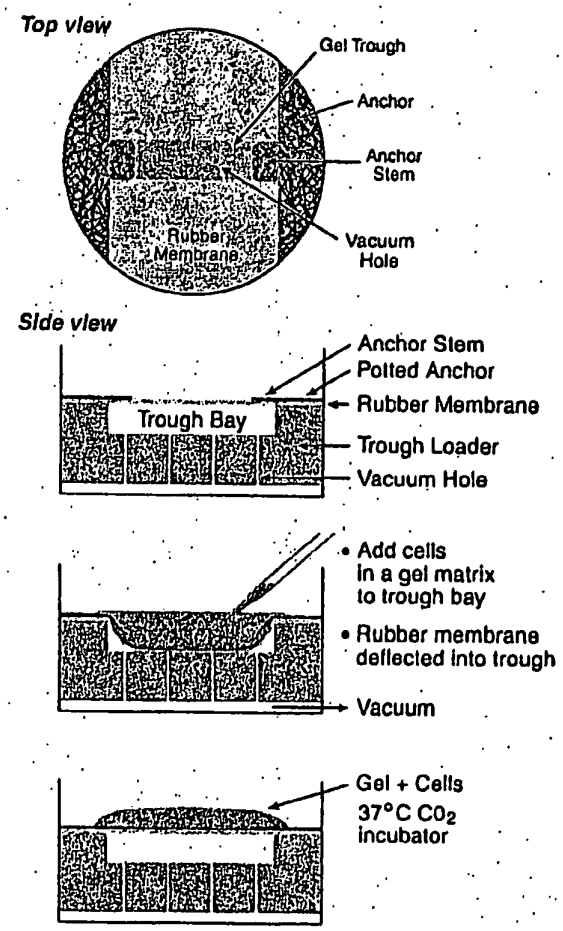


Figure 1. Specimen preparation

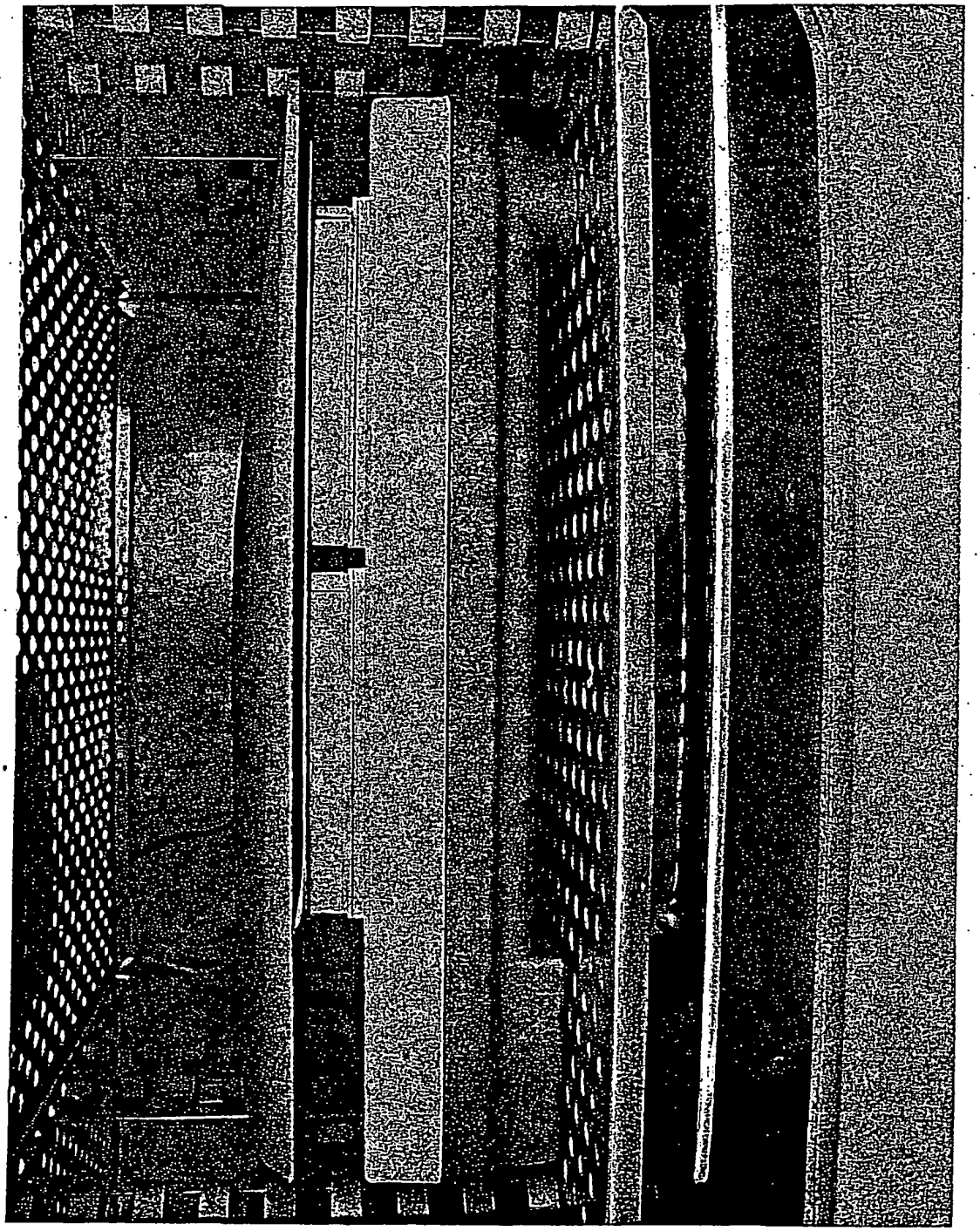

Figure 2: Culture plates prepared for scanning in the incubator.

Fig. 29

# MATERIALS and METHODS

- Using the custom program, ScanFlex™ (Flexcell International Corp., patent pending), the scanner was configured to collect images every hour for the first 4 hours of each day and every 2 hours for the remainder of the day, for 4 days.
- Culture plates were only removed from the incubator once/day to change the medium.
- Images were imported into SigmaScan software to quantify the area of each gel at each time point.

Fig. 30

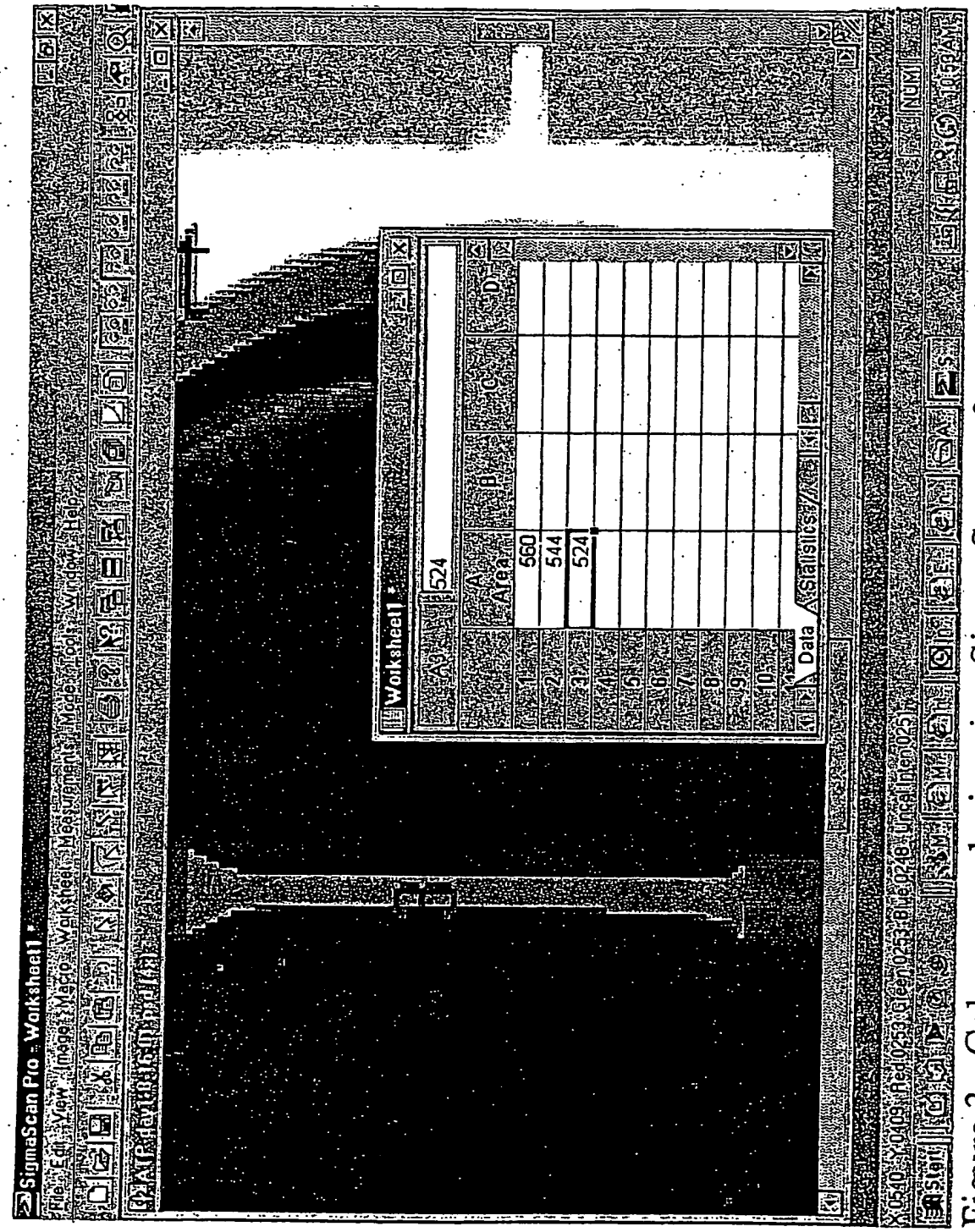


Figure 3. Gel area analysis using Sigma Scan software.

Fig. 31

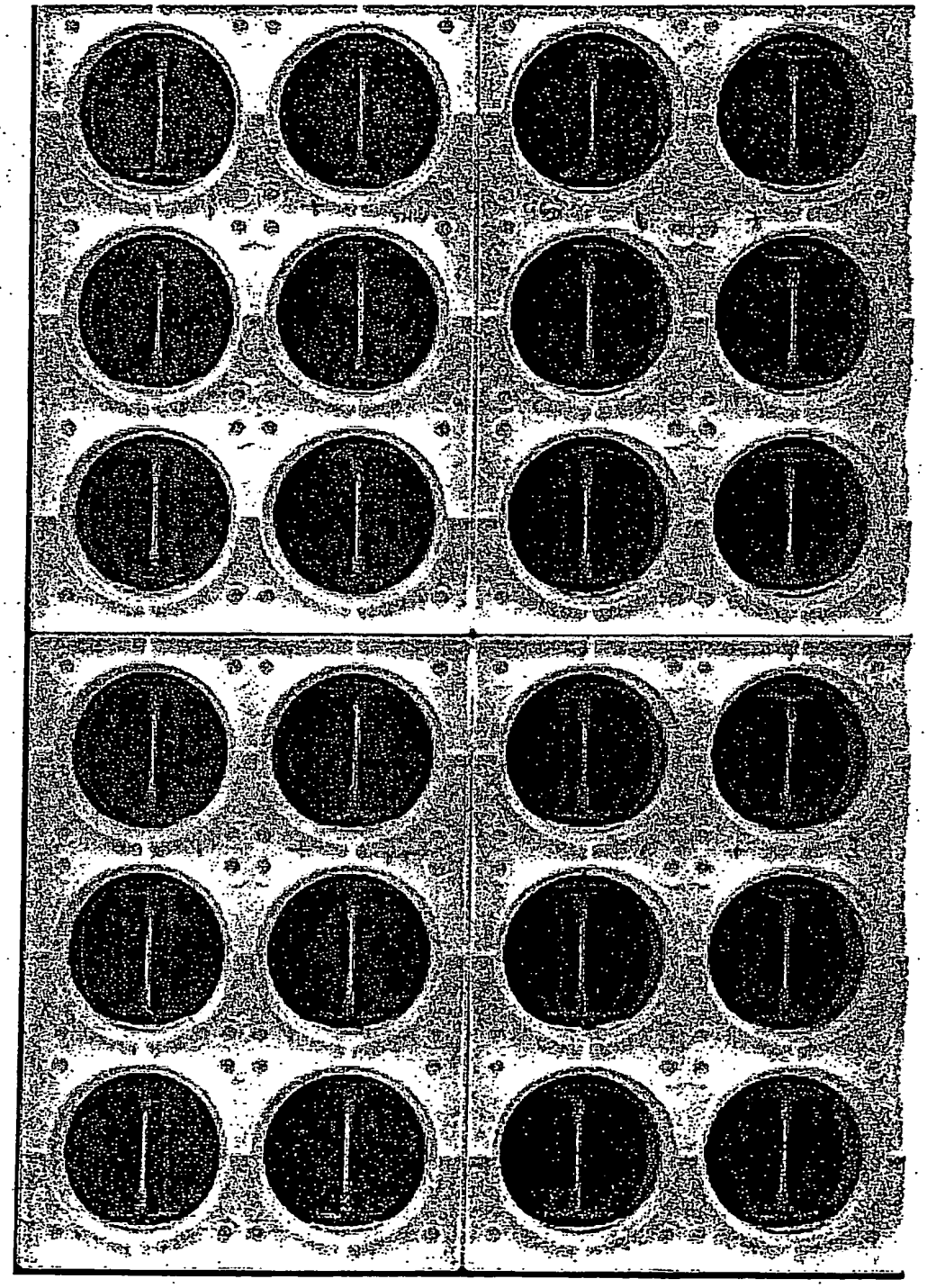

Figure 4. Scan of four Tissue Train™ culture plates taken in the incubator.

Fig. 32

# RESULTS

Gels experienced the greatest rate of contraction (58.6%) in the first 24 hours, and continued contracting to a total of 73.6% by the end of Day 4.

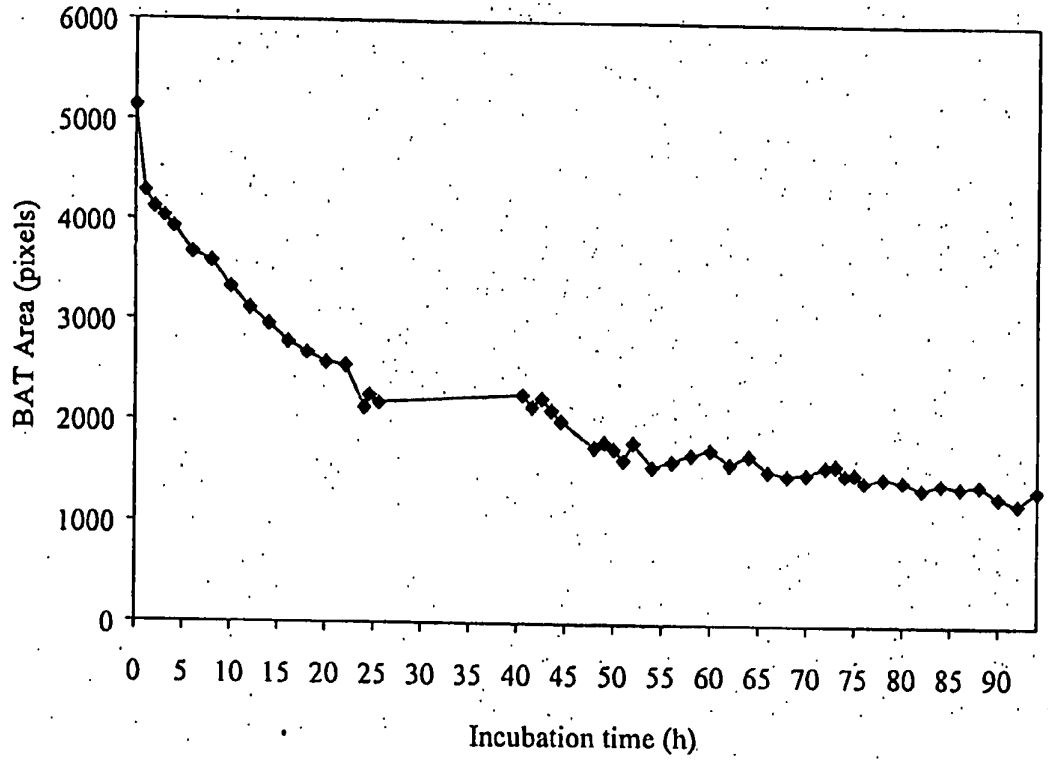


Figure 5. Contraction curve for gels from time of plating through day 4.

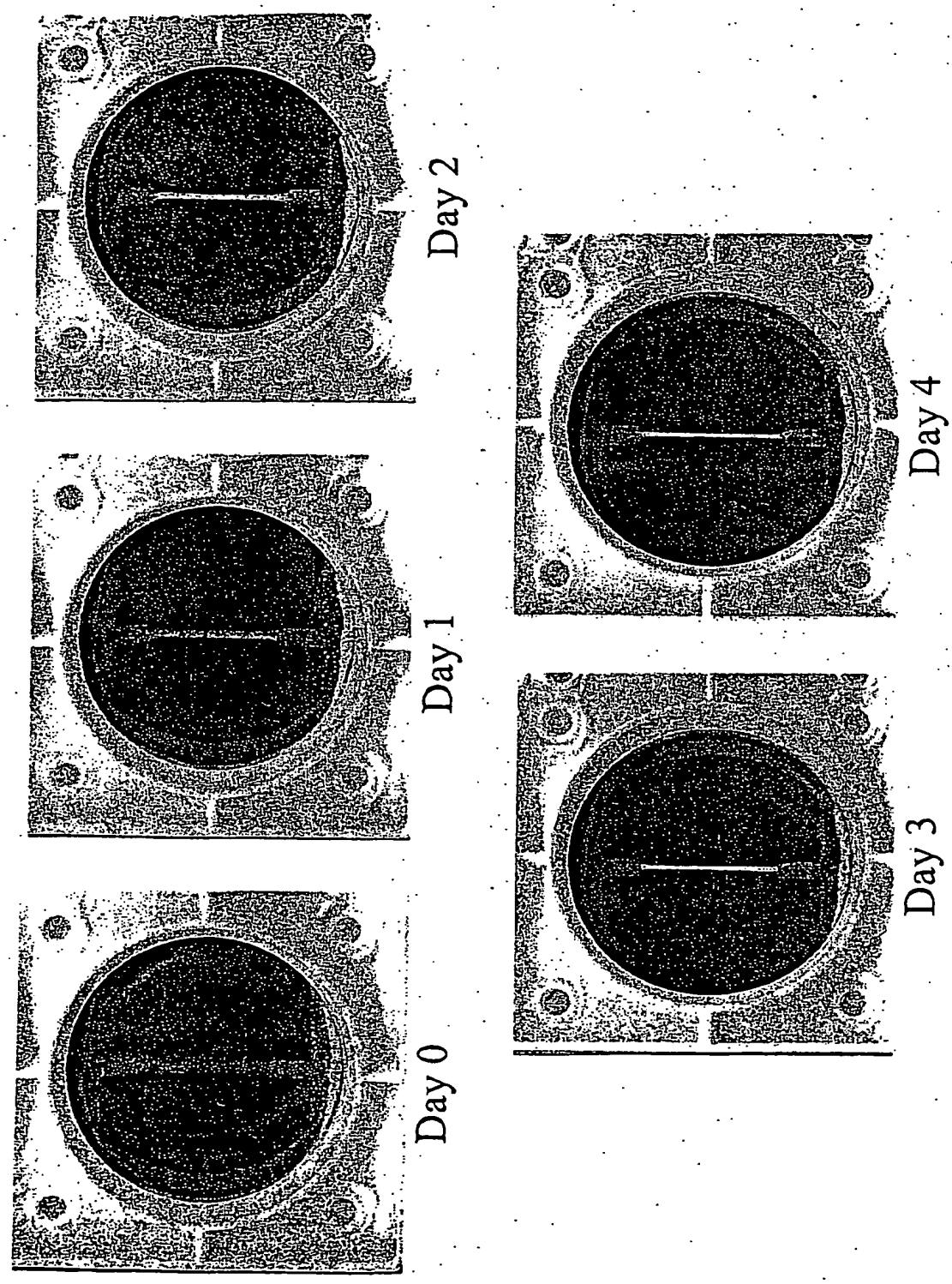


Figure 6: Specimen contraction from time of plating through Day 4.

Fig. 34

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